

CONTRIBUTIONS TO MARINE BIOLOGY

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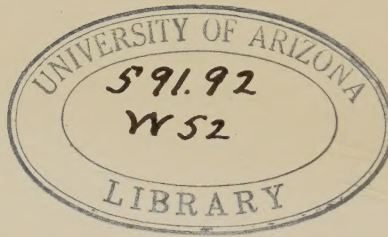
Lectures and Symposia Given at the Hopkins Marine Station,
December 20-21, 1929, at the Midwinter Meeting
of the Western Society of Naturalists

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PREFACE

The twenty-three papers comprised in this volume were read at the mid-winter meeting of the Western Society of Naturalists at the Hopkins Marine Station, December 18-21, 1929. Those by Professors Charles A. Kofoed and T. Wayland Vaughan constituted evening lectures, while the remaining twenty-one papers were presented at six half-day symposia and so are herein grouped under their respective symposium captions.

The title of this volume, "Contributions to Marine Biology," indicates a major interest of the Western Society of Naturalists, whose membership is composed largely of scientists residing in the Pacific seaboard states. This interest has of late years been rapidly increasing, owing to the exceptional opportunities which the several marine stations of the Pacific Coast afford for investigating throughout the entire year the many urgent problems of the ocean and ocean life. In recognition of this growing interest, the Western Society of Naturalists has instituted mid-winter meetings which have been devoted largely to work accomplished and in progress on the biology of the sea.

The importance of these contributions and their extensive scope will at once become apparent to the reader. The contributors are qualified to write authoritatively on the subjects selected and each has spared no pains in making his particular paper representative of his best efforts.

Moreover, it will be evident from the many and diverse topics here discussed how extensive and ramifying are the problems having to do with the life and conditions of life in the sea. Indeed, no limits can be set. The solution of these problems is dependent upon data and methods borrowed from every domain of science. Geologists, physicists, chemists, hydrographers, meteorologists, and many others, no less than biologists, must all contribute their share. As a consequence, the customary fields of science lose their confines. Through the requirements of advancing knowledge, for any given investigation the essential significance of any fact or method, whatever its source, is measured by what it may contribute toward the solution of the problems at hand.

The thanks of the Society are hereby tendered to Stanford University Press for assuming the task and the financial responsibility of publishing this volume. Especial thanks are due Professor William Hawley Davis, Editor of Stanford University Press, for his generous and untiring supervision of this publication from the very beginning. It is to be hoped that this first volume may prove to be of sufficient interest and worth to the scientific world to warrant the publication of similar volumes from year to year.

STANFORD UNIVERSITY
CALIFORNIA

C. V. TAYLOR
Secretary, Western Society of Naturalists

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I. FACTORS IN THE EVOLUTION OF THE PELAGIC CILIATA, THE TINTINNOINEA

BY CHARLES ATWOOD KOFOID

Professor of Zoölogy, University of California

The façade of the Prussian Marine Biological Station on the Island of Heligoland bears in enduring mosaic this inscription from Goethe's *Faust*:

Alles ist aus dem Wasser entsprungen,
Alles wird durch das Wasser erhalten.
Ocean gönn uns Dein ewiges Walten.

As biochemists, physiologists, botanists, and zoölogists, each of us might wish, in the interests of scientific accuracy, to reduce the sweeping comprehensiveness of the first two propositions of this motto by some limiting modifications. On the other hand, as biologists we all gladly recognize the imperial sway of the sea and join the procession seaward. We shall find ourselves in illustrious company, with Aristotle, with Darwin and Huxley, with Balfour and Van Beneden, with Haeckel and Dohrn, with Agassiz and Brooks, with Murray and Hjort, and with Whitman and Jordan.

All these and many other inquiring minds have been inducted into the charm and mystery of life in the sea and have sought the solutions of its many inviting problems in the study of its varied and manifold organic types and of their marvelously adaptive structures and functions.

It is fitting, perhaps it is even inevitable, that biologists should thus turn to the sea and that they should find there both adequate material for investigation and the intellectual satisfaction resulting from its analysis. The reasons for this are many and varied. Some are obvious, others are too subtle for analysis, too little comprehended for statement, or too elusive for expression. Perhaps an enumeration of some of the more obvious ones will serve to introduce my theme for this evening.

The sea is the oldest ecologic niche on the globe, provided the hypothesis of the permanence of the ocean basins is correct. Its changes in temperature and salinity, if any considerable ones have occurred since Pre-Cambrian times, must have been slight and in any event very gradually made. Time for the progress of evolution has thus been available longer in the sea than on land and certain important environing factors have remained more uniform in the sea than on the land.

The sea is also the largest arena on the globe in which the struggle for existence has been enacted and life in many of its varied forms has waxed and waned. Mere size of the environment in itself has, it is true, little direct biological significance to the individual, but indirectly it makes possible the increase and maintenance of great numbers of individuals in

which evolution proceeds and thus provides the quantitative background for diversification of the race.

The sea also has always presented a wide geographical range from equatorial to polar conditions, thus affording the complete range in temperature and in light. There has also been the great vertical range in depth, with its extreme conditions of temperature, light, and pressure. Not the least important among the geographical factors operating in the evolution of life is the seashore, with its tidal amplitude and its alternating contrasts of exposure and submergence, its mechanical shocks of breakers and of eroding currents. The varied forms of substrate from the beach to the edge of the continental shelf afford the stage for the evolution of attached and burrowing forms of life. The tributary rivers with their burdens of silt, their dilutant effects upon the salinity, and their varying contributions of chemical substances in solution, present new problems for adaptation and a grouping of environmental factors different from those of the land, as do also the land-locked brine-pools with their increasing salinity.

The sea also presents an aspect in all of these major factors of temperature, light, pressure, salinity, movement, and substrate, of gradual and regular change as over against the more abrupt and more strikingly irregular changes so characteristic of the environments of life upon land. Temperatures change slowly from latitude to latitude, from surface downward, and between noon and midnight. There are few submarine forests to interrupt the penetration of light, and pressure accumulates downward with little interruption by other factors as compared with that due to atmospheric conditions on land. The movements of the water, except at the immediate surface and in the breakers and tidal rips, are leisurely as compared with those of streams on land.

In consonance with these equable aspects of the marine environment we find the life of the sea delicately attuned to small environmental changes. Vertical migrations adjust the vertical distribution to changing conditions of illumination. Changes in the sizes and content of hydrostatic vacuoles, and adaptive modifications in the relative surfaces of the floating organisms of the plankton make adjustments in adaptation to the varying problems of flotation that arise with modifications in viscosity of the water following upon changes in temperature.

The well-known delicacy of pelagic organisms and the consequent difficulties of keeping them under laboratory conditions illustrate practically the narrow limits of this attunement of pelagic life to its environment as compared with that of the littoral fauna. To the pelagic organisms the shock of such sudden changes is unknown, but to the inhabitants of the littoral zone, and especially of the land, adaptation to extreme changes is the secret of survival.

The sea has been the stage whereon has been enacted the greater part of the drama of emergent animal life. The arthropods and higher vertebrates are the only outstanding exceptions to this limitation, and even these contain striking examples of elaborate adaptive flares among groups which have reverted to the marine environment, as for example seals, whales, petrels, and albatrosses.

The fundamental patterns of animal structure as represented in the main phyla have all had their origin in the sea. Some greatly diversified groups such as Radiolaria, Dinoflagellata, Foraminifera, sponges, corals, siphonophores, Copepoda, Trilobita, Brachiopoda, Cephalopoda, and Ophiuroidea have gone through their evolutionary flare in the sea.

The investigator may therefore approach the sea with confidence that it will throw light upon problems of the evolution of life.

It will be of interest to turn our attention this evening to a survey of the results of this evolutionary process in a single group of typical pelagic organisms, the Tintinnoinea, pelagic marine ciliates, which have had almost their entire evolution in the sea. These animals belong to the Protozoa, the most primitive phylum, and also belong to the more highly differentiated *Heterotrichida*.

This suborder Tintinnoinea is represented in a revision just completed (see Kofoed and Campbell, 1929) by 12 families, 51 genera, and 705 species. Of these genera only 3 have entered the fresh-water habitat, and only 8 valid species have been recognized therein. Their main area of evolution has thus been the sea. Not only is this true, but they have evolved principally in the high seas, only about ten per cent of the species appearing to be restricted to coastal regions. They therefore represent typical pelagic evolutionary results.

The material upon which our presentation is based is that of the Agassiz (1906) Expedition to the Eastern Tropical Pacific of 1904-1905 of the U.S.S. "Albatross," supplemented by the plankton collections of the same vessel in San Francisco Bay and in other Californian and Alaskan waters, by my own collections in a traverse of the North Pacific and Indian Oceans from Seattle to Colombo, Ceylon, and by the plankton collections off San Diego by the Scripps Institution.

A systematic revision of the entire group has recently been completed (Kofoed and Campbell, 1929) in the course of which the entire literature has been examined and the all-too-often conflicting and complicated systematic accounts of genera and species have been put in order. A better understanding of the mode of formation of the lorica or house in which the animal lives (see Campbell, 1926, 1927) which is the basis of classification, has made possible a clearer differentiation between modifications due to excess or lack of lorica-forming substance and those structural features of genetic significance.

Owing to previous lack of precision in generic distinctions and to varying concepts of species limits, a bewildering permutation of generic and subgeneric allocations of various species has often been made by different or even by the same investigator. One species may appear in five or six different genera, and not only trinomial but even quadrinomial usages in nomenclature have emerged.

Our own extensive material has made possible a clearer understanding of generic characters and of specific limits. In the light of these we have revised the entire group, combining under one specific name mere form variants of the species resulting from the amount of material available for the lorica or arising from the effect of temperature upon size. We have given specific status to all categories having valid structural characteristics, eliminating wholly all varieties and subspecies.

The absence of sharply defined barriers and the cosmopolitan, or, perhaps one should say, cosmo-thalassic, distribution of pelagic organisms in all seas of comparable environmental nature make the recognition of geographical races or subspecies so arbitrary a procedure within the Tintinnoinea that we exclude their recognition entirely. For example, *Tintinnus birictus* in the warmer parts of its area of distribution has smaller loricae than those in the colder parts, but these extremes in size intergrade. It is also possible that there may be an intergradation vertically in size between loricae formed in the upper and warmer and those formed in the deeper and colder levels of distribution.

In order that comparisons both within and between species may be made, many thousands of drawings have been prepared to provide a basis for measurement and analysis of both inter- and intra-specific differences. All species in all genera have also been drawn to the same scale of magnification and grouped together in order to bring out the interrelations of species within the genus, and to represent visibly the known results of speciation within each genus.

The results are published by the permission of Hon. Henry O'Malley, United States Commissioner of Fisheries, and of Dr. Thomas Barbour, Curator of the Museum of Comparative Zoölogy of Harvard University. Acknowledgments are made to the Board of Research of the University of California for grants in aid.

The Tintinnoinea are trumpet-shaped *Heterotrichida* with a leiotropic, spiral membranelle zone of highly developed cilia used in locomotion, in capture and selection of food, and in shaping up the secretion which forms the lorica.

The accompanying figure exhibits the details of structure. Note should be made of the relatively large size of these motor organelles and of their location and reach with reference to the size and structure of the lorica or house.

Since the entire classification of the Tintinnoinaea is based upon the structure of the lorica, its mode of formation is of interest. No account of the actual formation of an entire lorica has been published, and only scattered observations on parts of the process are available. These leave many lacunae in our knowledge and we are obliged to build up our picture of the process from incomplete data.

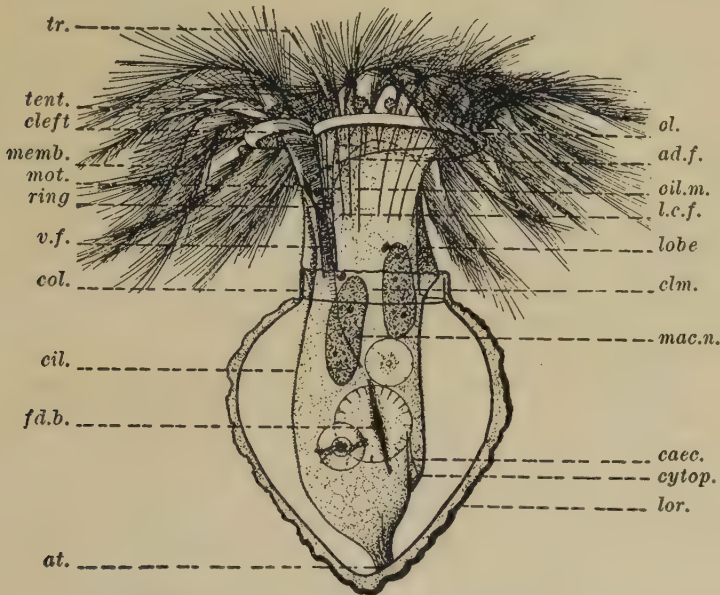


FIG. 1.—Semi-diagrammatic figure of *Tintinnopsis* showing the general morphology of the cytostome. *ad.f.*, adoral fiber of the neuromotor apparatus; *at.*, attachment to the lorica; *caec.*, caecum; *cil.*, somatic cilia; *cil.m.*, ciliary membrane; *cl.*, peristomal collar; *clm.*, column; *cleft*, cleft in the peristomal margin; *col.*, lorical collar; *cytop.*, cytophyge; *fd.b.*, food body; *lobe*, lateral column lobe used in shell-building; *lor.*, outlines of the lorica; *l.c.f.*, lateral ciliary field; *mac.n.*, macronucleus; *memb.*, membranelle; *mot.*, neuromotorium; *ring*, circumoesophageal ring; *tent.*, tentaculoid; *tr.*, trichocysts; *v.f.*, ventral fiber of the neuromotor apparatus. $\times 850$. After Campbell (1926, p. 189, Fig. B).

All Tintinnoinaea build a lorica or house in which they live permanently, although they will detach their adhesive stalk and leave the lorica in adverse conditions. The available evidence indicates that a new lorica is formed only at binary fission and only for the anterior daughter, the old house being retained by the posterior daughter.

The lorica is formed from a substance secreted by the parent and stored up in granules in the cytoplasm in its anterior end near the mouth. This substance is extruded from the mouth of the anterior daughter in the late stages of plasmotomy following upon the divisions of the macro- and

micronuclei and the formation of a new adoral zone for the posterior daughter. Possibly this is preceded by the division of the neuromotorium and the posterior migration of its posterior moiety to the side of the parent body. About this as a center the new adoral zone forms laterally and later becomes the anterior end of the posterior daughter.

The accompanying figures (Figs. 2-5) show the accumulation and localization of a deeply stainable substance which in preffission stages of *Favella franciscana* is progressively segregated in the oral region of the anterior daughter. In Figure 2 the secretion is dispersed; the macronuclei have not as yet divided. In Figure 3 the granules are segregated immediately below the surface on the side where the new adoral zone is emerging. In Figures 4 and 5 the granules are massed adjacent to the mouth of the anterior daughter. In the last figure plasmatomy is progressing, though the new adoral zone is on the under side and not figured.

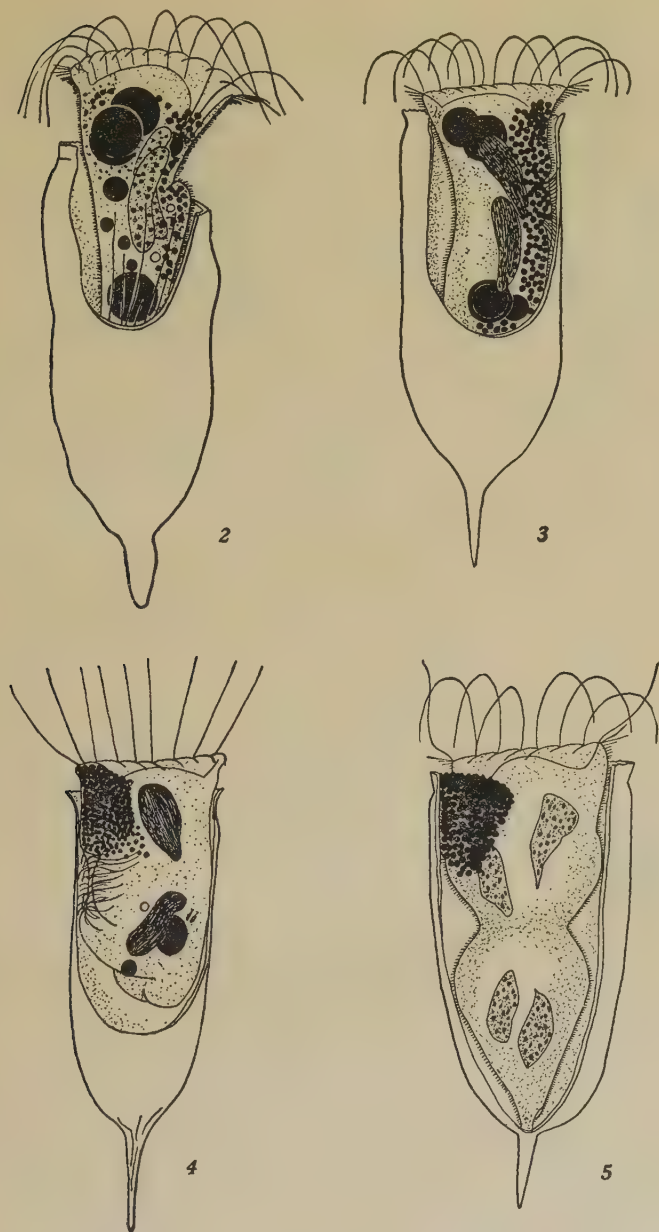
Additional information on the later stages in the formation of the lorica is given by Schweyer (1909) whose three figures (our Figs. 6-8, p. 8) show three stages in the formation of the lorica of *Tintinnus apertus* Kofoid and Campbell (as *T. inquilinus* O.F.M.).

From these figures it appears that the secretion (not shown in the figures of the body of the animal) is poured out at or near the level of the mouth of the anterior daughter. Binary fission has progressed so far that the adoral zone of each daughter is fully developed. The action of the ciliary mechanism, in conjunction with rotation of the body, in the formation of the collar in Figure 7 and of the larger ring of substance in Figure 8, is suggested by the form and location of the partially formed lorica. In Figure 6 the secretion immediately surrounds the anterior adoral zone as a cap or lies in concentric shell-like fragments above as though molded by the successive rotations of the adoral zone and hardened *in situ*. It is significant that even these fragmentary and imperfect formations have taken on the same alveolar structure as that of the parent lorica.

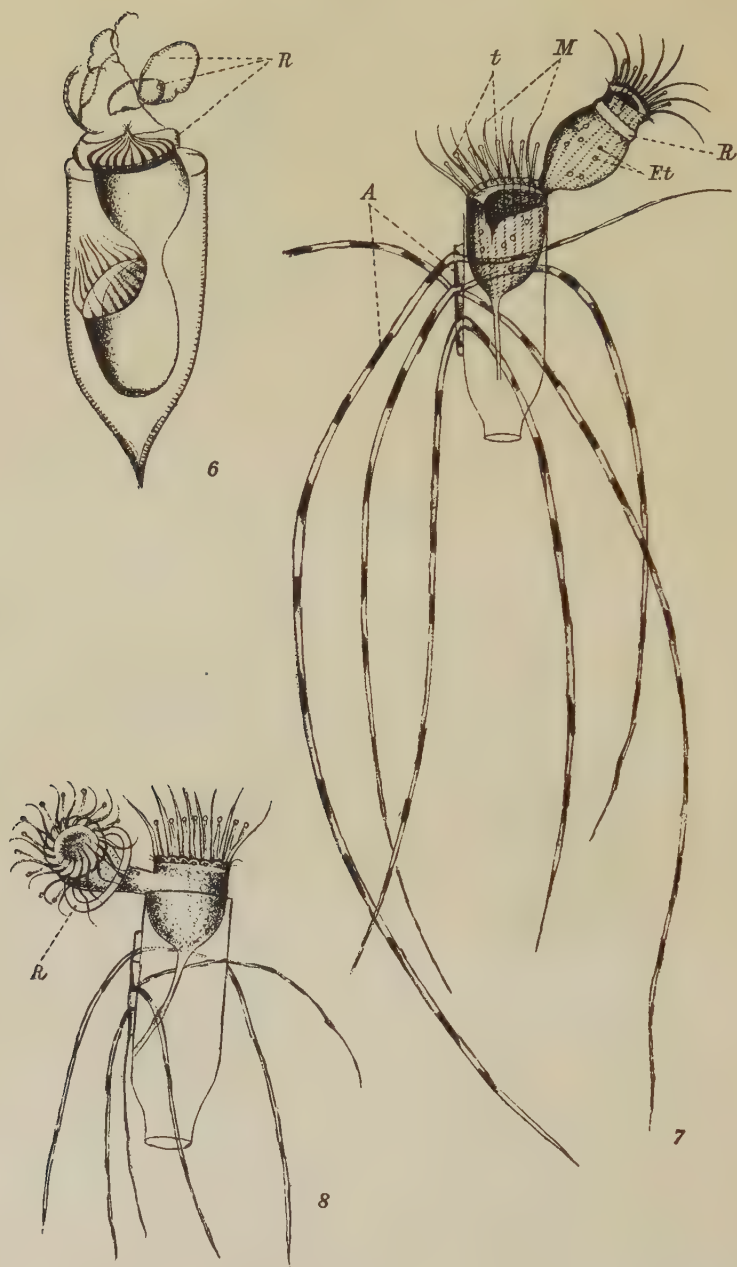
From the imperfect knowledge of the actual process of the formation of the lorica and from the comparative study of the various patterns of loricae formed in the Tintinninea we can arrive at certain conclusions regarding the lorica and its systematic evolutionary significance.

The substance of the lorica is a secretion of the parent animal prior to binary fission. Loricae are therefore formed only at binary fission and only for the anterior one of the two daughters. It appears to be formed but once in the life of the individual. The secretion of lorica-forming substance has been observed only in animals in which the preparations for binary fission are also apparent.

The substance is chitin, or chitinous in nature, swells up rapidly on contact with sea water, and hardens quickly with the formation of pri-



FIGS. 2-5.—Successive prefission stages in *Favella franciscana* $\times 200$. After Campbell (1927, Fig. C, 1, 5, 6, and 7).



FIGS. 6-8.—Binary fission and formation of the lorica in *Tintinnus apertus* Kofoid and Campbell. After Schweyer (1909, Pl. 10, Figs. 4, 6, and 8). The lorica in Figs. 7 and 8 is attached to the frustule of a pelagic diatom *Chaetoceros*. *A*, bristle of *Chaetoceros*; *Ft.*, fat droplet; *M*, membranelle; *R*, ring, or other anlage of lorica; *t*, tentacle.

mary, secondary, and tertiary reticular or alveolar structure of varying degrees of fineness in the different genera. There is often formed a distinct outer and inner lamella between which the secondary and tertiary alveoles are arranged in prismatic fashion.

There are very great differences in both the coarser and finer patterning of the substance which suggest a wide range of chemical and physical properties of the lorica-forming substances among the different genera. Thus in *Tintinnidium* (Fig. 29, 4*) and in *Tintinnopsis* (Fig. 12) the



FIG. 9.—Loricae of the species of *Parundella*. 425–446. $\times 200$. (The numbers, such as 425–446, in this and other figures, are those of Kofoid and Campbell, 1929.)

lorica often carries a ragged coat of surplus blobs of alveolar substance and the shape in patterning is more or less chaotic. In *Brandtiella*, on the other hand, there are two regions, an inner one of definite shape and an outer more amorphous mantle. In genera such as *Parundella* (Fig. 9) the wall is homogeneous, bilamellate, translucent, and shows scarcely any trace of visible alveolar structure. In *Climacocylis* (Fig. 10, p. 10) the secondary alveoles are very coarse, rather loosely aggregated, very delicate in consistency, and extraordinarily transparent. The physical nature of the substance of the lorica appears to be a generic or family character, in some instances at least.

The lorica is not always entirely composed of the secreted substance but may incorporate foreign objects within its substance as an enveloping matrix. These seem to be derived from two sources, primarily and prin-

* See p. 35. This number and others thus cited are as in Kofoid and Campbell, 1929, Literature Cited, p. 38.

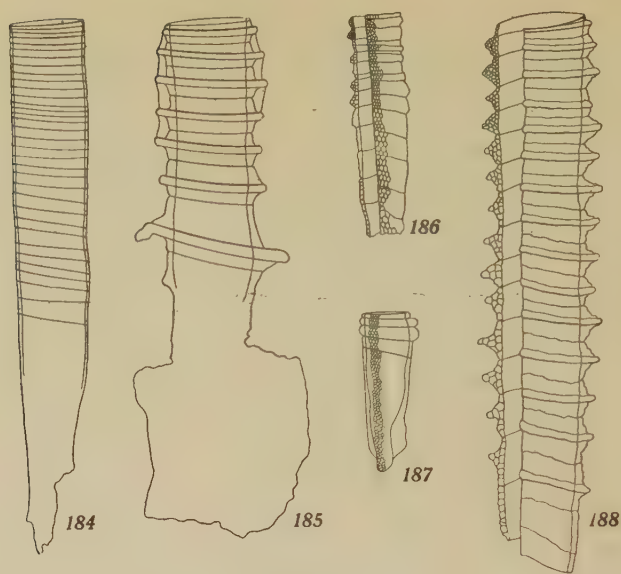


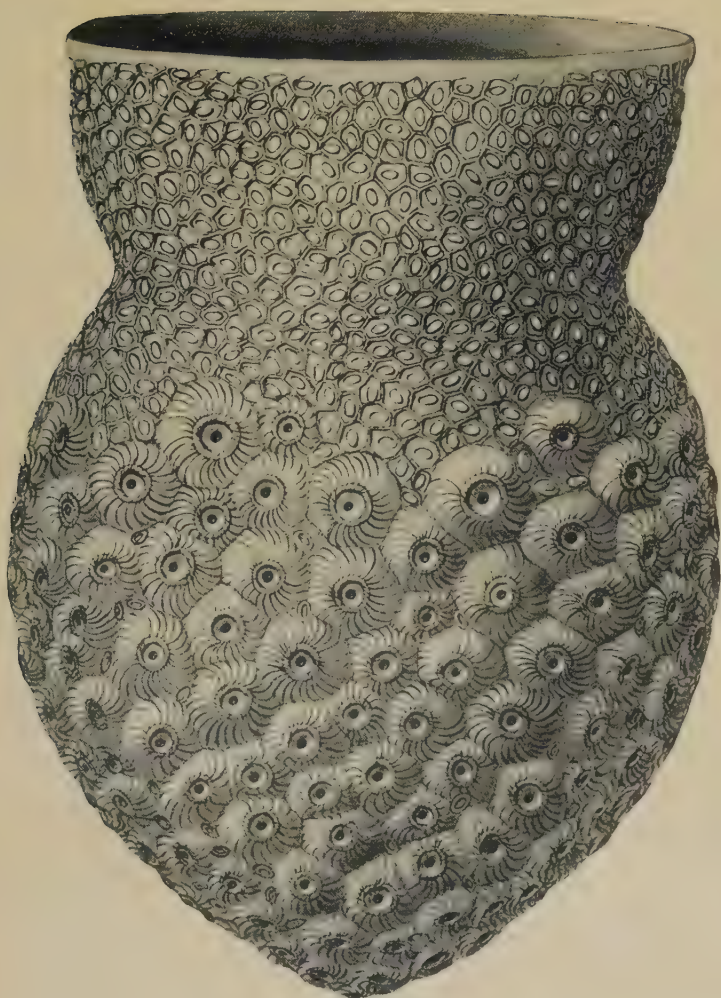
FIG. 10.—Loricae of the species of *Climacocylis*. 184–188. $\times 200$.

cipally from undigested fecal residues, largely the calcareous coccoliths from the flagellate family Coccolithophoridae. Many of the Tintinnoinea feed largely upon these organisms of the nannoplankton, and their curious buttons, disks, ellipsoids, etc., are built into the pattern of the lorica as in *Codonella* (Fig. 11 and Plate I) in designs comparable with those of the houses of fresh-water rhizopods which utilize the frustules of diatoms in their houses, except that the coccoliths are even more distinctly built into the wall of the lorica than are the diatoms in that of the rhizopod house.

In other cases, especially in fresh-water and neritic species growing in silt-laden waters, the lorica tends to agglomerate to itself, possibly only during its formative period, not only blobs of lorica substance but also minute particles of foreign matter, which assist in giving the ragged appearance to the loricae of such a neritic genus as *Tintinnopsis* (Fig 12, p. 12). The extent to which fecal residues and foreign agglomerates are utilized in the formation of the lorica and the manner in which they are utilized also appears to be something of a generic character. Genera with homogeneous substance, such as *Proplectella* (Fig. 13, p. 13) and *Undella* (Fig. 14, p. 13) use neither coccoliths nor agglomerates, while genera with patterned loricae such as *Codonella* (Fig. 11) and *Dictyocysta* (Fig. 15, p. 14) contain species which habitually make extensive use of coccoliths in the wall of the lorica.

The lorica of each species is the resultant of two factors, namely, the chemical and physical nature of the secreted substance and the stereotyped

PLATE I



Lorica of *Codonella acuta* Kofoid and Campbell, from Station 4666 in the Peruvian Current. $\times 1440$.

FIG. 11.—Loricae of the species of *Codonella*. 98–132. $\times 200$.

behavior of the organism in the very brief period of the molding and hardening of this substance in the newly forming lorica.

This brings us to the most difficult and perplexing part of the problem in the analysis of the formation of the lorica, namely: Is the new lorica formed in entirety by the anterior daughter which occupies it, or do both daughters share in this function? If the former supposition is true, how can the anteriorly located membranelles shape up the elaborately differentiated aboral end of the lorica into a pedicel, knob, skirt, and lance in such species as those of *Xystonellopsis* (Fig. 16, p. 15), and how are spiral striae which cover the entire lorica developed? If the latter is the case, we are confronted by the extraordinary fact that the most conspicuous character of the species, namely, the patterning of the lorica, is the result of the stereotyped behavior of the *two* daughter individuals. Moreover, this

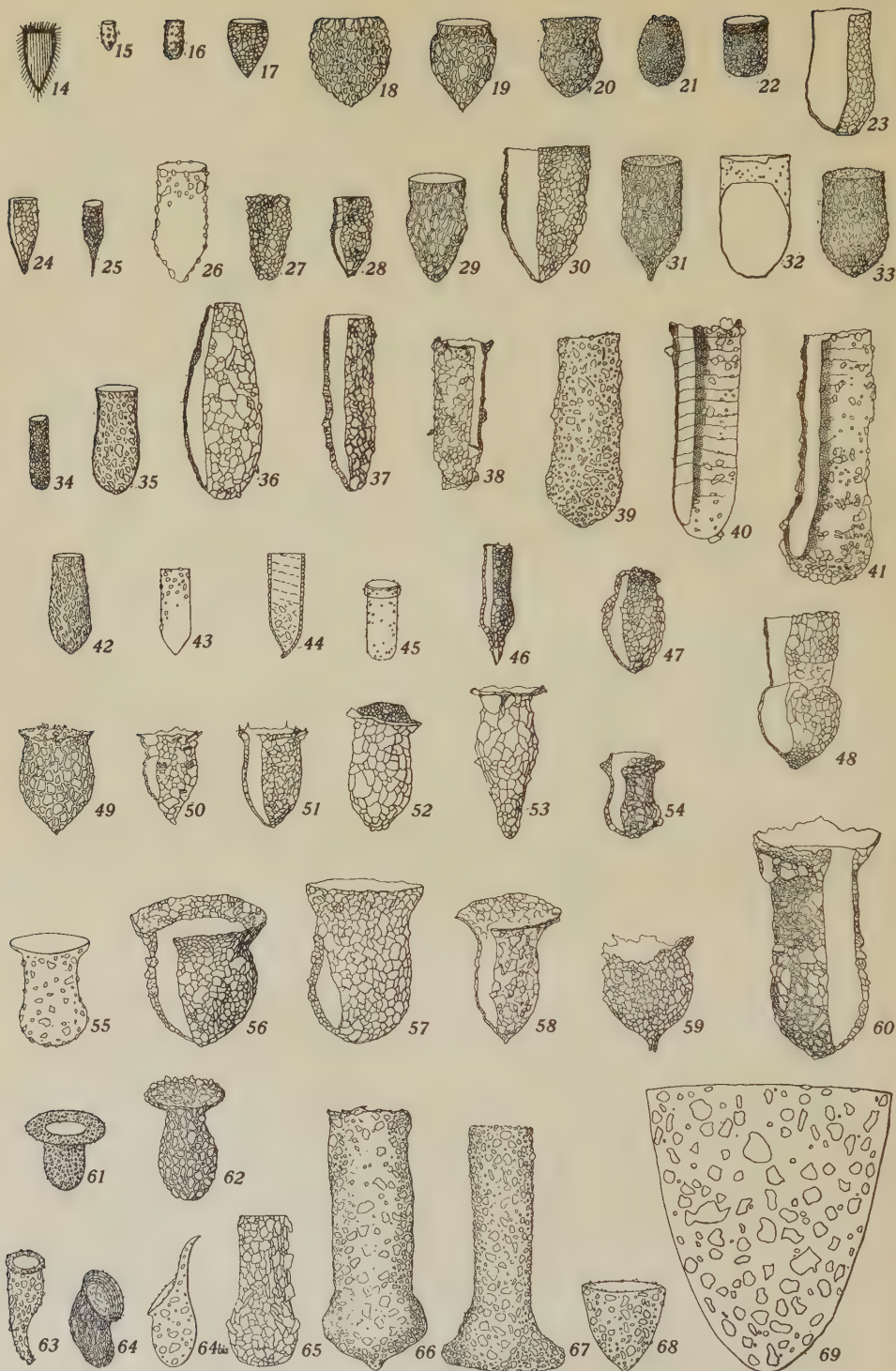


FIG. 12.—Loricae of species of *Tintinnopsis*, 14-69, $\times 200$.

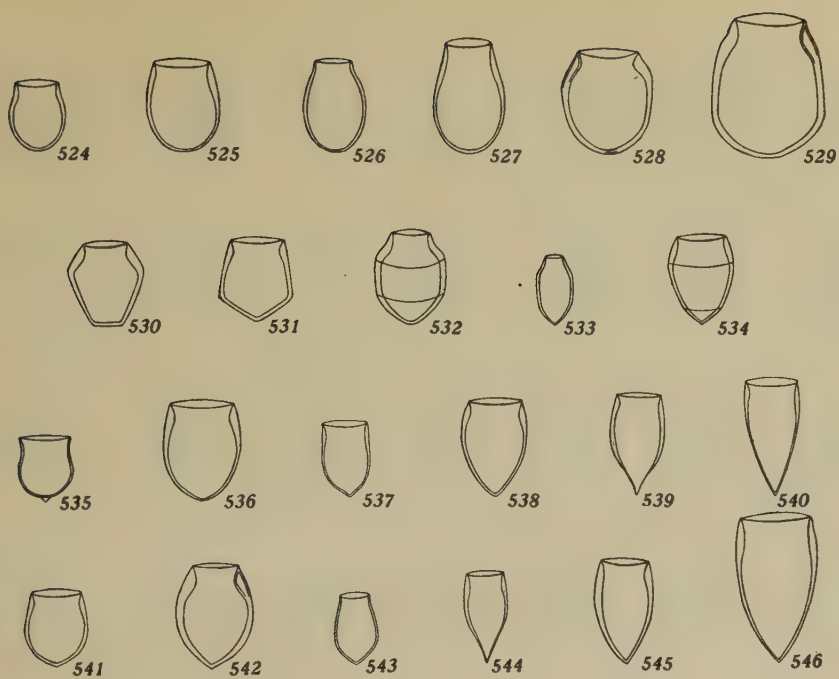


FIG. 13.—Loricae of the species of *Proplectella*. 524–546. $\times 200$.

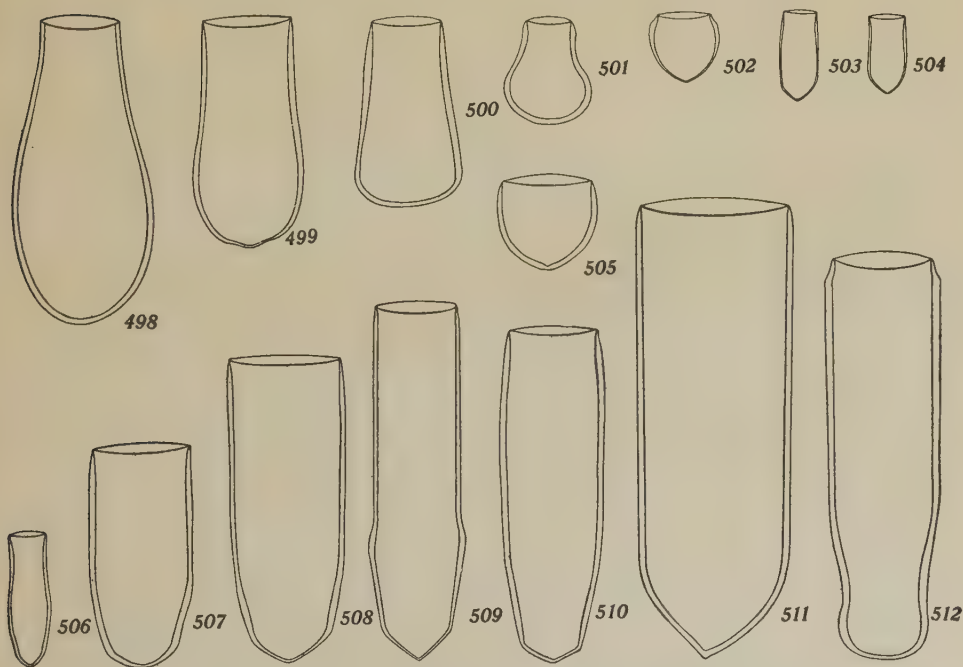


FIG. 14.—Loricae of the species of *Undella*. 498–512. $\times 200$.

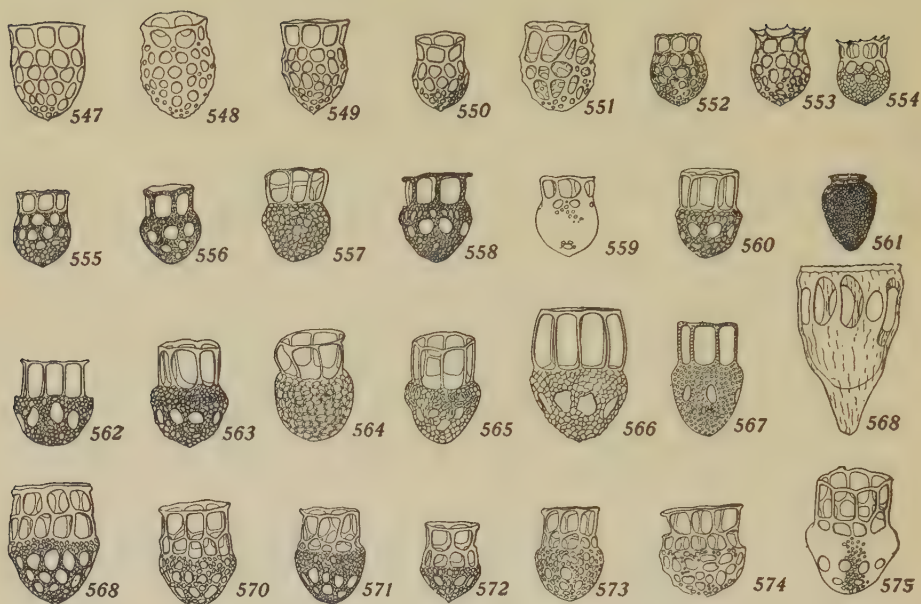


FIG. 15.—Loricae of the species of *Dictyocysta*. 547–575. $\times 200$.

diagnostic behavior is evinced only at the initial stage of their existence and in fact while they are in the very act of severing their protoplasmic continuity. To complicate the problem still more we have to face the perplexing situation that the anterior daughter will mold the anterior portion of the lorica, while the posterior one would shape up the aboral end. Since the two ends are so obviously very different in structure, it follows that the stereotyped and instinctive behavior must be very different on the part of the two collaborators.

The grounds upon which the hypothesis that both daughters share in building the lorica rests are three: First, the difficulty which the anterior daughter would have in reaching the aboral end. Secondly, the fact that there are two main regions of structural differentiation in the evolution of the types of loricae of the Tintinninea, the oral and the aboral. Lastly, both regions exhibit the structural effects of spiral action, of rotation, and both ends show the results of a tapering-off process, the anterior in the narrowing down of the spiral lamella of the lorica, as in *Coxiella* (Fig. 29, 203), and in the collar of *Codonellopsis* (Fig. 18). In the aboral end, on the other hand, the tapering-off process results in the widespread appearance in many genera of species with an aboral point, horn, or lance, as in *Xystonellopsis* (Fig. 16).

In this connection attention should be directed to observations on the behavior of the living animal in its house. It is attached by a more or less

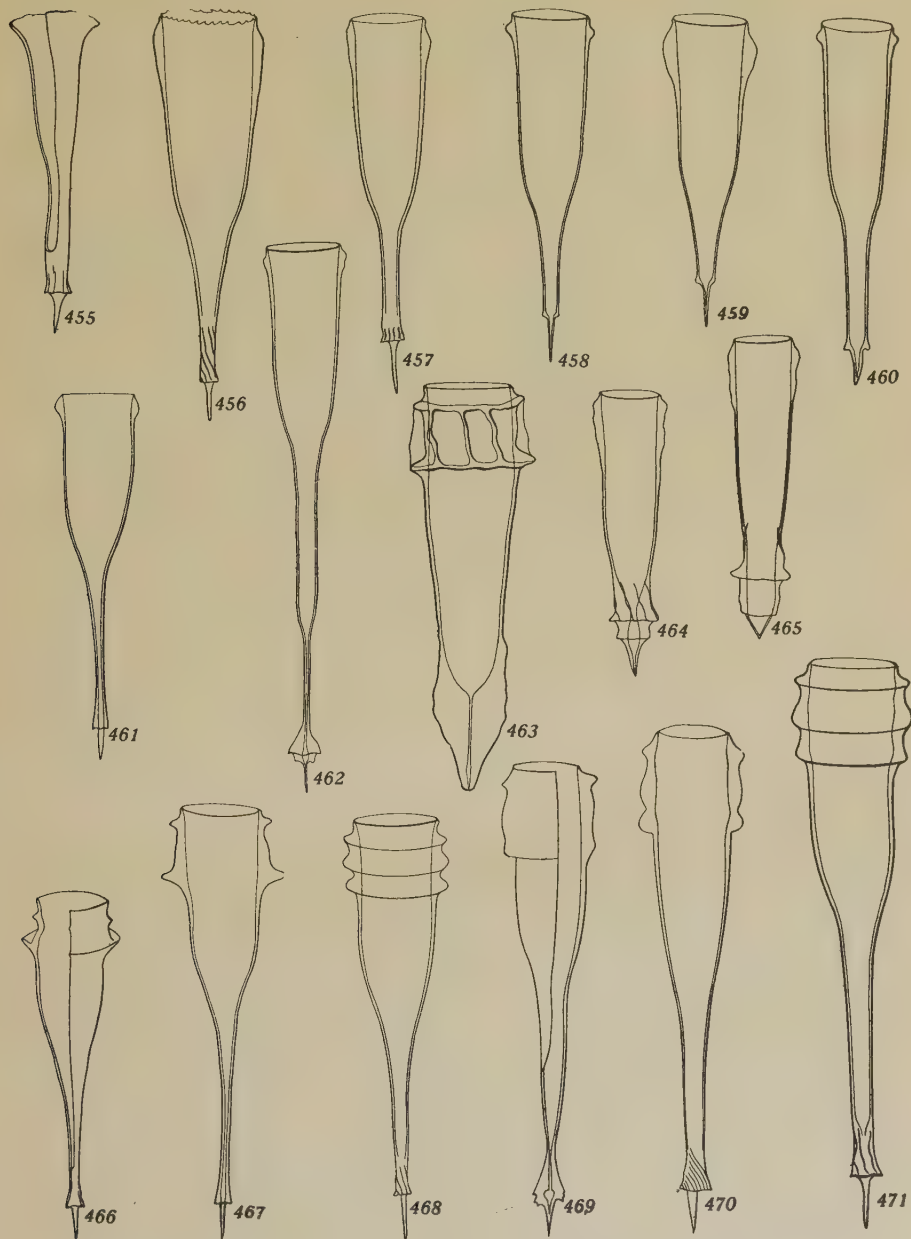


FIG. 16.—Loricae of the species of *Xystonellopsis*, 455–471. $\times 200$.

slender pedicel with an adhesive, spreading, terminal foot adherent to the bottom or side of the lorica. The body can be greatly elongated and extended beyond the oral rim of the lorica. The membranelles are long and very flexible. The action of the membranelles sets the lorica rotating about its long axis. The body also can and does rotate independently of the rotation of the lorica, in which case the attenuated stalk is progressively twisted.

The effect of this spiraling rotation is visible in the loricae (Fig. 17). There are two regions in which this occurs, often independently of one another. In some genera, as in *Climacocylis* (Fig. 10) and *Coxliella* (Fig. 29, 203), the whole lorica is formed by the laying up of a spiral ribbon, often tapering in width as the oral rim is approached. In such loricae the method of formation seems to be a fairly uniform outpouring of the secretion during a period of uniform spiral rotation of the anterior daughter, with a diminishing flow in the last few turns.

In loricae such as those of *Codonellopsis* (Fig. 18, p. 18), in which there is a dense, thick-walled, spiral bowl surmounted by a spiral, hyaline, thin-walled collar, it appears that there must be two types of secretion, a massive, continuous flow while the bowl is being shaped, followed by a period of scanty flow of a thinner secretion and more evidence of spiral activity while the collar is being formed. The varying number of turns in the collar in different loricae of the same species suggests marked variations in the amounts of the material available both within the species and among different species for the formation of the collar. Thinning out of the last turns occurs also in these structures.

Spiral structure also appears at the two ends of the lorica, suggesting independent action of the two daughters as in *Favella brevis* and *F. helgolandica* (Fig. 19, p. 19) where an oral spiral lamina of a few turns is coincident with a spiral twist to the wings on the aboral horn.

Spiral structure is in most species of some genera, as in *Salpingella* (Fig. 31, 673), *Xystonellopsis* (Fig. 16), and *Steenstrupiella* (Fig. 31, 593), limited wholly to the aboral region, and would seem to result from the behavior of the posterior daughter alone.

An entirely different type of structure appears in the superficial ribs, striae, and fins found on the outer surface of the bowl and running longitudinally or in a more or less marked, usually leiotropic but sometimes dextrotropic spiral over the whole or only a part, usually the aboral, of the bowl. Such structures are found in the striae of *Protocymatocylis*, *Cymatocylis* (Fig. 29, 188), and *Rhabdonellopsis* (Fig. 30, 421); the spiral shelf of *Climacocylis* (Fig. 29, 188) and *Xystonella scandens* (Fig. 30, 454); and the more or less fin-like elevations on *Amphorella* (Fig. 31, 592), *Amphorellopsis* (Fig. 31, 594), *Odontophorella*, *Dadaiella* (Fig. 31, 613), *Ormosella* (Fig. 31, 633), *Stelidiella* (Fig. 31, 627), *Daturella* (Fig. 31,



FIG. 17.—Spiral structure in the loricae of the Tintinnoinea. $\times 125$. 76, *Tintinnopsis cincta*; 158, *Codonellopsis indica*; 183, *Laackmanniella prolongata*; 185, *Climacocylis scalaria*; 204, *Coxiella helix*; 209, *Helicostomella subulata*; 291, *Favella helgolandica*; 375, *Metacylis annulata*; 406, *Rhabdonella anadyomene*; 416, *Rhabdonella lohmanni*; 423, *Rhabdonellopsis minima*; 454, *Xystonella scandens*; 470, *Xystonellopsis torta*; 485, *Xystonellopsis krämeri*; 664, *Daturella stramonium*; 679, *Salpingella altiplicata*; 689, *Salpingacantha ampla*; 696, *Epicranella bella*.

662), *Salpingella* (Fig. 31, 673), *Salpingacantha* (Fig. 31, 695), and *Epicranella*. The numerical limits, position, and distribution of the structures suggest the agency of the membranelles of one or of both daughters in applying, arranging, or otherwise determining their pattern.

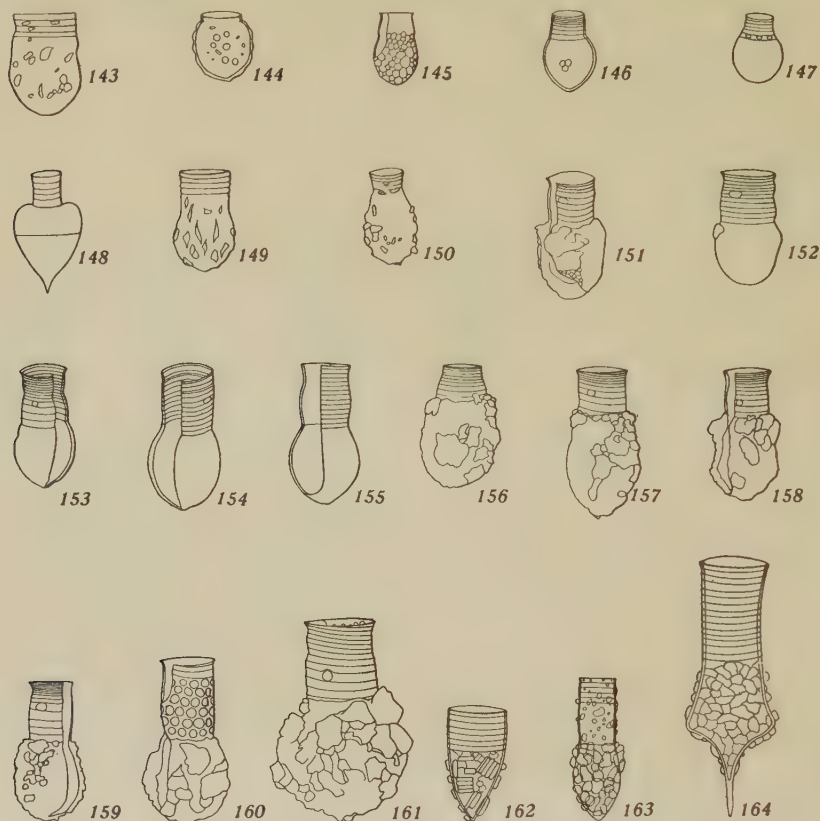


FIG. 18.—Loricæ of species of *Codonellopsis*. 143–164. $\times 200$.

The two major regions of structural differentiation of the lorica are the suboral and the aboral areas, contiguous, respectively, to the membranelles of the anterior and posterior daughters. In the suboral region are found such structures as the crest, ledge, trough, suboral thickening of the wall, one or more suboral rings, nuchal constriction, nuchal shelf, and various funnel-shaped modifications of the anterior region designated as the collar.

In the aboral region are to be found, below the bowl, the pedicel, knob, skirt, aboral horn or lance, and various additions to these in the form of striae or fins. It seems more probable that the membranelles and oral

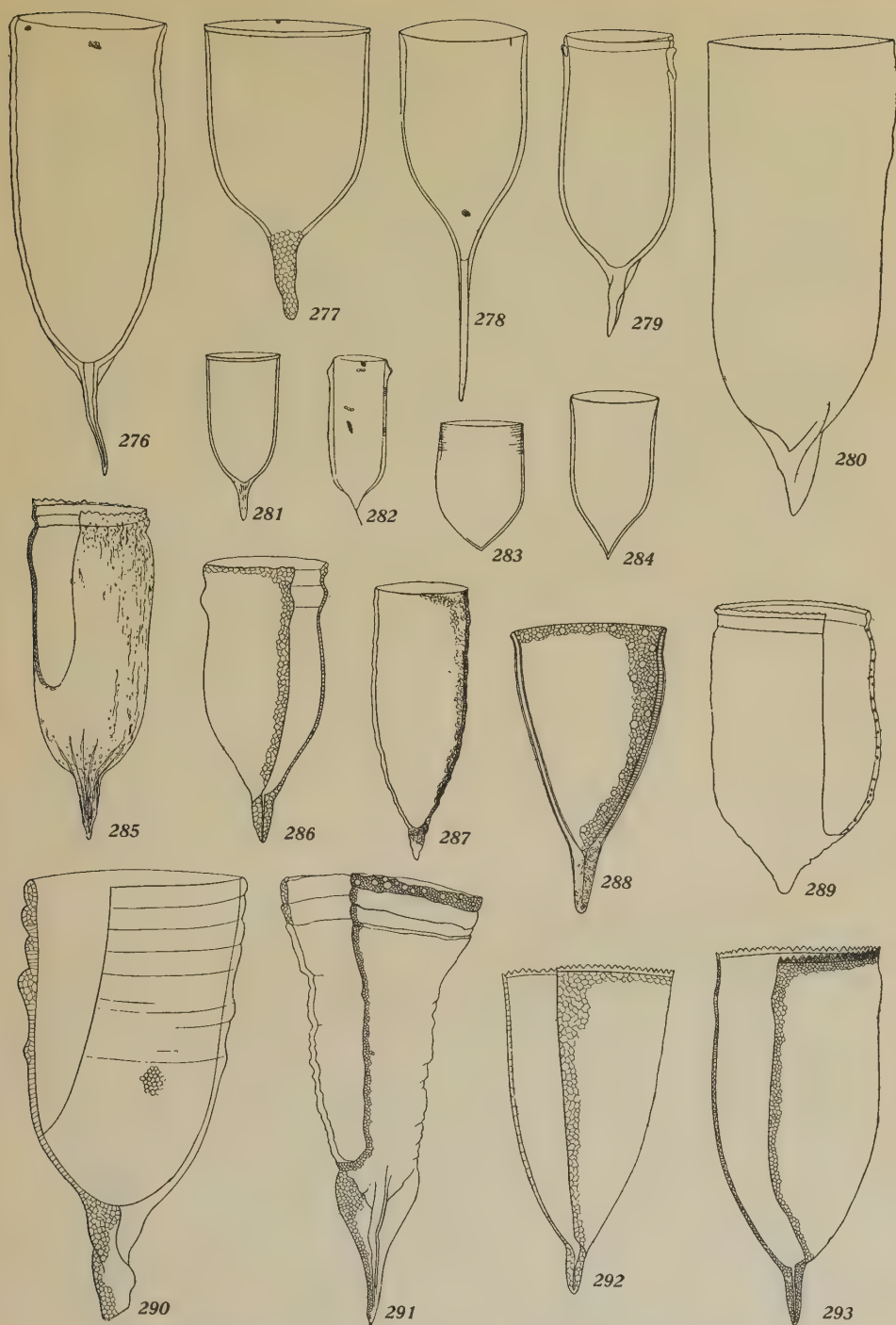


FIG. 19.—Loricae of the species of *Favella*. 276-293. $\times 200$.

cavity of the posterior daughter have shaped these structures than that the anterior daughter could do it, especially since the spiral structures are so often present. The presence of a narrow canal through the aboral horn in *Codonellopsis* (Fig. 18) suggests the persistence of the protoplasmic strand between the two daughters, while the broadly rounded aboral end of the bowl, or the prolongation of the bowl in the aboral horn are suggestive of a finishing-off action by the membranelles and the oral region of the posterior daughter after plasmotomy has been completed.

The repetition of rings on the bowl may be due to recurrent waves of an accelerated discharge of the secreted material without corresponding acceleration of the molding action resulting in local thickening or in buckling, as in the undulations of the wall of *Undellopsis* (Fig. 20) or the repeated suboral rings of *Xystonellopsis* (Fig. 16).



FIG. 20.—Loricae of the species of *Undellopsis*. 513–523. $\times 200$.

The relatively simple, hyaline loricae of such genera as *Tintinnus* (Figs. 27, 28) and *Proplectella* (Fig. 13) lack entirely all structural differentiation at both ends, except such as arise from slight differences in slope, thickness, flare, and attenuation.

The outstanding feature in all the speciating process and in generic differentiation among the Tintinnoinea is the play of behavior, of movements of the scarcely as yet differentiated daughters, in the distribution of the secreted material, and in its shaping into the diverse generic patterns and into the superposed, less differentiated specific patterns. This behavior involves a certain degree of co-ordination on the part of the two daughters, each with its own distinct line of activity, beginning with the period in which they are still attached and, in most genera at least, continuing beyond the period of protoplasmic discontinuity for at least a short time.

The function of the lorica or house of the Tintinnoinea is problematical in the extreme. Two major uses suggest themselves, protection and flotation. Protection against most of their carnivorous foes seems futile in the light of the fact that many plankton feeders, such as *Salpa*, take their catch with little selection, straining all organisms from the water within certain limiting dimensions, and making no choice among them. The locomotor powers of the housed ciliate are also very considerable and would facilitate a certain degree of escape from animals seeking to capture particular species as food.

Flotation, on the other hand, is a common necessity of all plankton organisms and is generally facilitated by increase of specific surface, or by hydrostatic vacuoles. In the ciliates the locomotor activities necessitate a certain density and continuity of the cytoplasm which militates against the latter method, while the absence of internal or external skeletal structures is unfavorable to extensions of the surface. The house within which the animal lives affords an increase in surface without modification of the shape of the body or change in the consistency of the cytoplasm for purposes of flotation, and this without much increase in volume.

The aboral end is forward in locomotion, and its pointed or rounded shape facilitates the morphologically backward progression, so that the house does not materially interfere with locomotion. It may be of significance that there are relatively few non-housed ciliates in the marine plankton as compared with that of fresh water, and that the evolutionary flare of the housed Tintinnoinea in the sea is enormous as compared with that of ciliates elsewhere. They constitute nearly forty per cent of the known ciliates, marine and fresh-water.

FACTORS IN THE EVOLUTION OF THE TINTINNOINEA

Any consideration of these complex problems must of necessity be highly speculative. Our objective data consist of the existing fauna, a total of 51 genera and 705 species, with structural groupings and geographical distribution which are the visible results of the evolutionary process. This array of evidence suffices to incite some comment on their relations to the various factors with which the orthodox evolutionist expresses his analysis of the process of speciation or evolution. Among these are isolation, natural selection, action of the environment, mutation, hybridization, orthogenesis, and, we may add, quanta.

The geographical distribution of the known species of the genera of the Tintinnoinea in the Eastern Tropical Pacific is characterized by the coincident occurrence of two or more species of a genus at many of the collecting stations. The same feature appears in the records of Brandt (1907) in the Atlantic and of Laackmann (1909) in the Antarctic. For example, in our records at Station 4724 in the South Equatorial Drift the

genus *Tintinnus* was represented by seven species in a surface collection made with a 14-inch net within the uppermost fathom of the surface. Intermediate hauls of the same net from 300–0 fathoms at the adjacent Stations 4723 and 4725 of the same current each contained only eight species of the genus. The accompanying map exhibits the distribution of the seventeen species of the genus *Tintinnus* occurring in the surface collections of the Eastern Tropical Pacific.

There is on the whole a very striking absence of any convincing or even suggestive data in the distributional evidence now available that the species of the genera of the Tintinnoinea are isolated in accordance with Jordan's Law, the operation of which is so frequently demonstrated in the areal isolation of the species and subspecies of land vertebrates. A map of the distribution of the species of any genus of the Tintinnoinea in the Eastern Tropical Pacific, or elsewhere, is dominated by coincident occurrence rather than by isolation. The available data are, however, critical only for surface collections. There is some evidence of vertical limitations for some species, in that a few of them have never been taken in the surface collections. Some species occur in surface collections at one station and only in the subsurface collections at the same or adjacent stations, a distribution which suggests a considerable vertical range.

The possibility of some degree of isolation by limited vertical distribution within the zone above the light floor is not wholly excluded. Our distributional data are based on records of the loricae, many of which are empty when found in the plankton. The animal either leaves the lorica in the rush of filtration in the ascending net, or on the application of chloretone or of the formalin used in fixing. It is possible also that empty loricae may last for some time after the death of the occupant. How long they can persist is unknown, but Antarctic genera such as *Cymatocylis* have not been found persisting as empty loricae in the Humboldt or in the Benguela currents emerging from the Antarctic region. It seems probable that empty loricae will, in the absence of the locomotor activity of a living occupant, rather quickly sink below the level of the light floor.

From the available data it seems evident that spatial isolation does not play any large part in the evolution of Tintinnoinea but that whatever isolation does exist must be of a different nature. It may be temporal, physiological, or behavioristic, but hardly spatial.

The action of natural selection can only be inferred from the adaptive features of structure in the loricae. If the lorica primarily serves a buoyant function, as we have suggested, adaptations in that direction have a selective value. Spiral ribs, fins, and the spiral shelf all tend to retard sinking, and delicate alveolation, as in *Climacocylis*, tends to reduce the overweight of the lorica. Rings and agglomerated particles likewise give increase of specific surface.

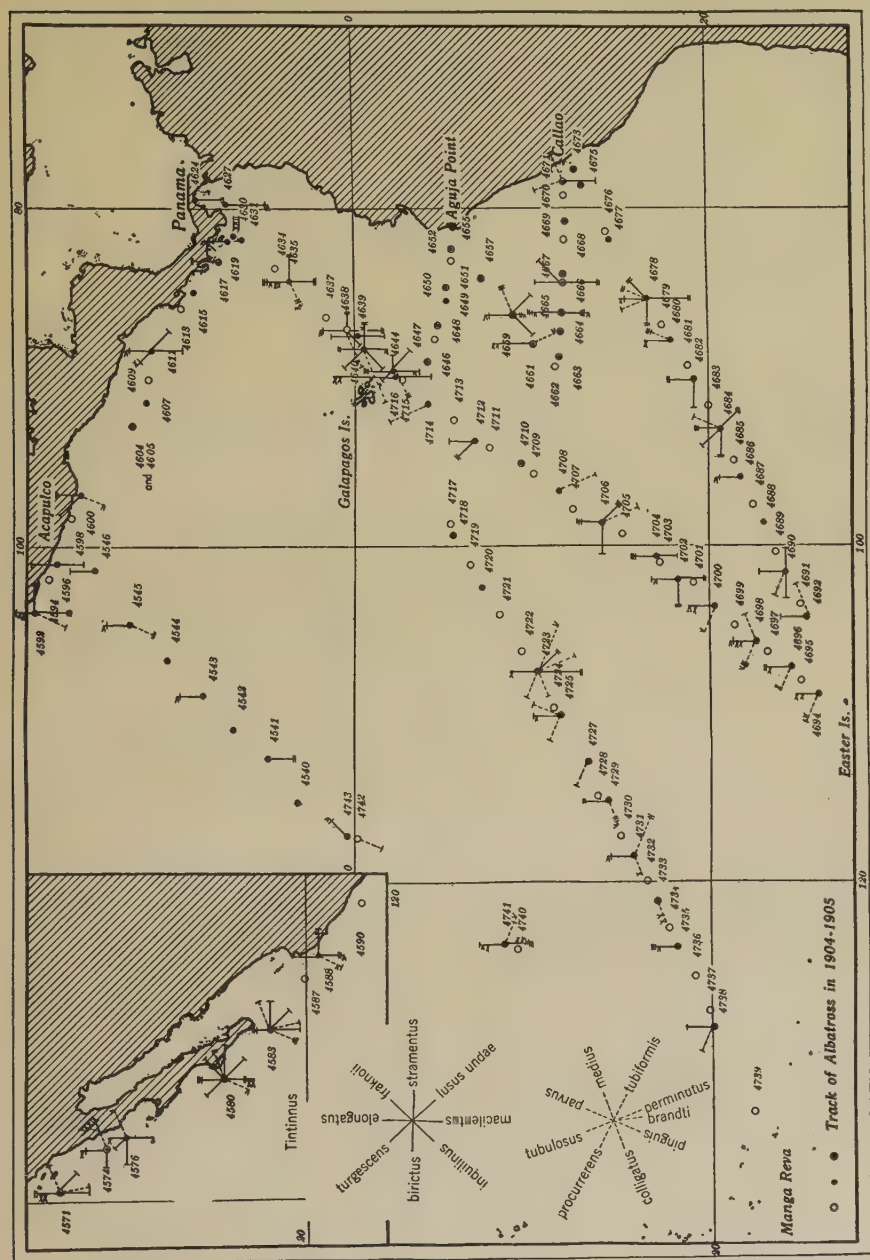


FIG. 21.—Map of the distribution of the species of *Tintinnus* in surface collections in the Eastern Tropical Pacific from the data of the Agassiz Expedition of 1904-1905. Station numbers are those of the U.S.S. "Albatross." A frequency-percentage of one per cent is indicated by a single radial line. A cross-arm on the ray is added for each additional one per cent. The number of radial lines corresponds to the number of species, and their positions indicate the species, as determined by the key.

Structures such as these are sometimes present in all members of a genus, and specific differences are more often made up of very minor modifications in size, proportions, angles, slopes, and the like. Their character and range are such that, if the interspecific differences have survival value, it is beyond comprehension why intergrading individuals should be eliminated or why one species differs in survival value from others in the same genus. All seem to lie within the dead-line of survival value, and so might many other mutant types. It is difficult to find satisfactory evidence for the differentiating survival value of the type of differences between the species. To say that these varied differences are all linked with unseen ones that do have such values is of no great assistance in the problem.

On the other side of the question there is the fact that only a relatively few of the 705 species are individually numerous. Many are very rare, as though lacking in reproductive vigor, though the house may exhibit no signs of the decrepitude of its maker.

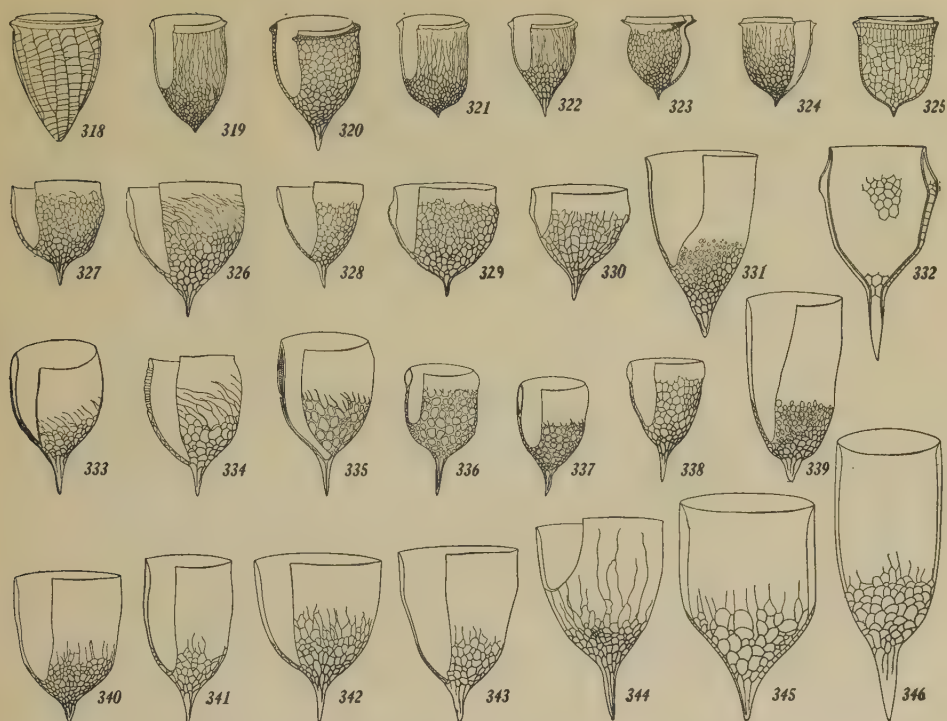
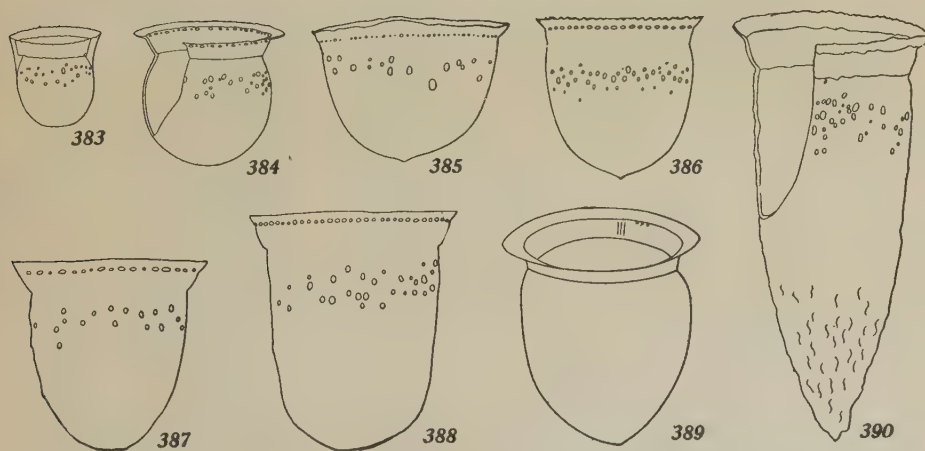
In the matter of generic distinctions there is considerable evidence of marked differences in the nature of the lorica-forming substances. The substance within each genus is fairly uniform. In some, as in genera of the Undellidae, the substance is homogeneous, does not form patterns, and the loricae are relatively free from structural differentiations of all kinds except rings, bulges, and pointed aboral ends.

In other genera, such as *Dictyocysta* (Fig. 15), *Epiplocylis* (Fig. 22), *Petalotricha* (Fig. 23), and *Ptychocylis* (Fig. 24, p. 26), there is in each a characteristic patterning of the surface with lines or reticulations suggestive of patternings directly referable to the chemical and physical natures of the differing colloidal substances. Differences such as these can hardly be regarded as brought about by natural selection or as incident thereto. They are the expression of definite chemical and physical differences, and their origin and pattern rest on chemical and physical laws.

The generic distinctions in the main in the Tintinninoidea usually involve these differences in the lorica-forming substance and are rather sharply marked. There is usually but little difficulty when one has all of the species before him in making satisfactory generic allocations of most species. In a few instances, as between *Tintinnopsis* and *Stenosemella*, the intergradation is close. Some large genera, such as *Codonella* and *Xystonellopsis*, are less coherent than others, such as *Dictyocysta*.

The possibility of hybridization is suggested by certain curious "cross-overs" of generic characters between quite distinct genera in the case of single species in one of the two genera concerned.

Thus *Xystonella scandens* (Fig. 25, 434, p. 26) is the only species in the genus *Xystonella* which has a spiral shelf. In all other features the lorica is that of a typical *Xystonella*. This shelf is the outstanding generic char-

FIG. 22.—Loricae of the species of *Epiplocyis*. 318–346. $\times 200$.FIG. 23.—Loricae of the species of *Petalotricha*. 383–390. $\times 200$.

acter of the genus *Climacocylis*. There is clearly an intermingling of the characters of these two genera in *X. scandens*.

In like manner *Xystonellopsis ornata* (Figs. 16 and 25, 463) of the

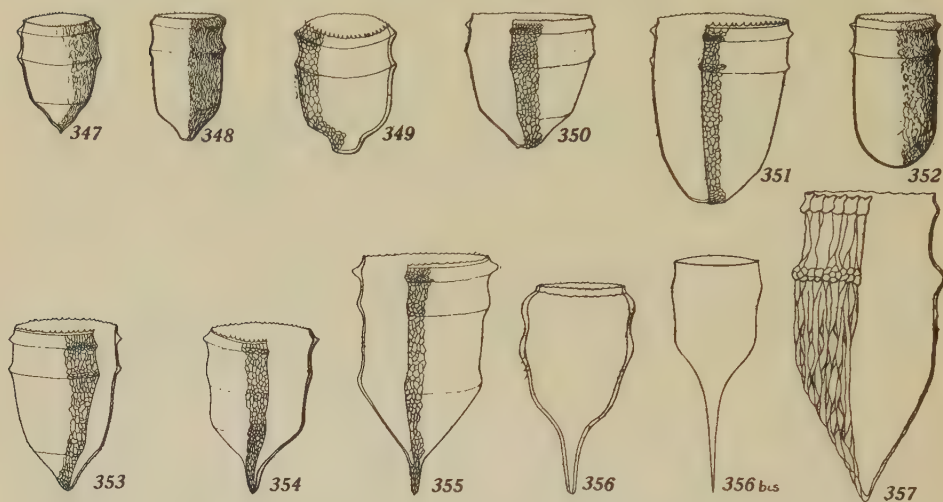


FIG. 24.—Loricae of the species of *Ptychocylis*. 347–357. $\times 200$.

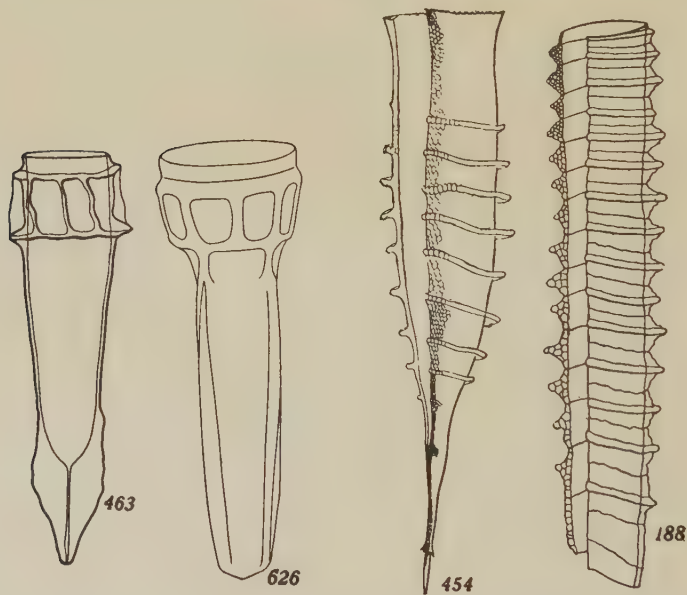


FIG. 25.—“Cross-over” of generic characters in the Tintinnoinea. 463, lorica of *Xystonellopsis ornata*; 626, lorica of *Stelidiella fenestrata*; 454, lorica of *Xystonella scandens*; 188, lorica of *Climacocylis elongata*.

family Xystonellidae differs from all other species in its genus in having vertical bars between the two suboral rings. In this respect it resembles the remote genus *Stelidiella* of the family Tintinnidae, the species of which have a rather stout aboral end. As compared with all but one other species in *Xystonellopsis*, *X. ornata* has an unusually stout aboral end. There is thus in this species also a curious admixture of characters of two rather widely separated genera. An origin of these admixtures by hybridization is suggested. There are some other instances of such admixtures of less striking character among the genera of the Tintinnoinea. The alternative hypothesis is convergent mutation.

In many of the genera there are instances of characters which are present in some species and absent in others in the genus. Thus the aboral horn or point is present in some and absent in other species in *Tintinnopsis* (Fig. 12) and in *Codonella* (Fig. 11). The aboral flare occurs in similar fashion in *Tintinnopsis* and *Leprotintinnus*, as though there were present the expression of recessive characters in some species of the genus and not in others. Many of the most prominent structural characteristics of the loricae are shuffled among the species of the genus in a fashion very suggestive of the play of Mendelian combinations.

In this connection the dependence of such structural features of the lorica upon the behavior of the animal is to be emphasized. The lorica is not an anatomical structure arising in development or by redifferentiation in the ciliate after conjugation or binary fission. It is the direct product of the mode of behavior of the animal at the particular moment of the formation of the lorica. An example of such differential behavior between species or genera is seen in the differences between the homogeneous structure, the spiral structure, and the annulate pattern of the lorica. In the non-spiral pattern the behavior does not lead to spiral lamination; in the spiral pattern the behavior during secretion leads to a continuous spiral, ribbon-like deposition of the substance of the lorica; and in the annulate pattern the secretion is intermittently and more or less regularly increased. The structure in each case is dependent upon behavior. The shuffled genes in such cases as this represent types of behavior of the animal rather than morphological patterns of structure primarily.

The relation of the environment to the evolutionary process appears to have certain rather striking correlations in the Tintinnoinea. They are not limited to this group but more or less affect all pelagic life.

The two greatest environmental contrasts in the marine environment are those of temperature contrast between equatorial and polar regions and between surface and deeper levels in the tropics, and between the high seas and the neritic area in matters of salinity and of amplitude and rapidity of the changes in chemical content, temperature, and movement of the water.

The structure of the lorica reflects the influence of these contrasted regions. The influence is evident both in generic groups and within the individual species. Temperature through its relations to viscosity of the water affects the buoyant action of the lorica. It also affects directly the velocity of all chemical reactions, and thus influences growth, reproduction, secretion, and possibly also the rate of formation of the lorica. Even a casual inspection of the dimensions of the loricae of genera limited, respectively, to polar and tropical seas reveals the fact that genera limited to polar seas such as *Cymatocylis* and *Parafavella* have a very much larger proportion of species of larger sizes than of smaller ones, and that tropical genera, as for example *Amplectella* and *Proplectella* (Fig. 26), are either wholly made up of small species or have very few large species.

A quantitative study of the dimensions of 1,000 individuals of *Tintinnus tenue* in the area explored by the Agassiz Expedition to the Eastern Tropical Pacific shows the loricae of this species taken in the Peruvian Current at stations of lower surface temperatures (66° to 74°F.) are predominantly larger than those taken elsewhere at stations where this temperature was 75° to 83°F. There is thus a parallelism between the type of modifications of the individual regarded as fluctuating and directly correlated with the action of the environment upon the individual, and those differences in size between species which we are wont to regard as hereditary and genetic. The correlation is with the same aspects of temperature in both types of differences, individual and genetic.

In one other important particular the evolution of the Tintinninea exhibits a correlation with the environment, namely, in the relation of temperature to the number of species. More species are found in equatorial regions than in polar ones. In our revision of the suborder we list 705 species. Of these, 8 are from fresh water, 59 are restricted to Arctic waters, over 40 to the Antarctic, and 515 to warm temperate and tropical regions. As a general rule the number of species taken by any one haul of the plankton net in circumpolar seas is small, but the number of individuals may be very great. On the other hand, in tropical seas the reversed relation exists. The species are numerous and the individuals of each are few, at least of the great majority of them.

The total of known species in polar waters is 101, and in the tropics 515. The polar faunas are not as well known as those of the tropics. These crude figures, however, give a ratio of 1 to 5 between the respective numbers of species in these contrasted regions. If the difference between the extremes of temperature in these two contrasted regions approximates 20°C. Van 't Hoff's Law calls for a quadrupling of the rate of chemical reactions and of biological processes, or a ratio of 1 to 4. In accordance with this law and the resultant higher rate of chemical changes within the living substance in warm seas we may expect an increase in the oppor-

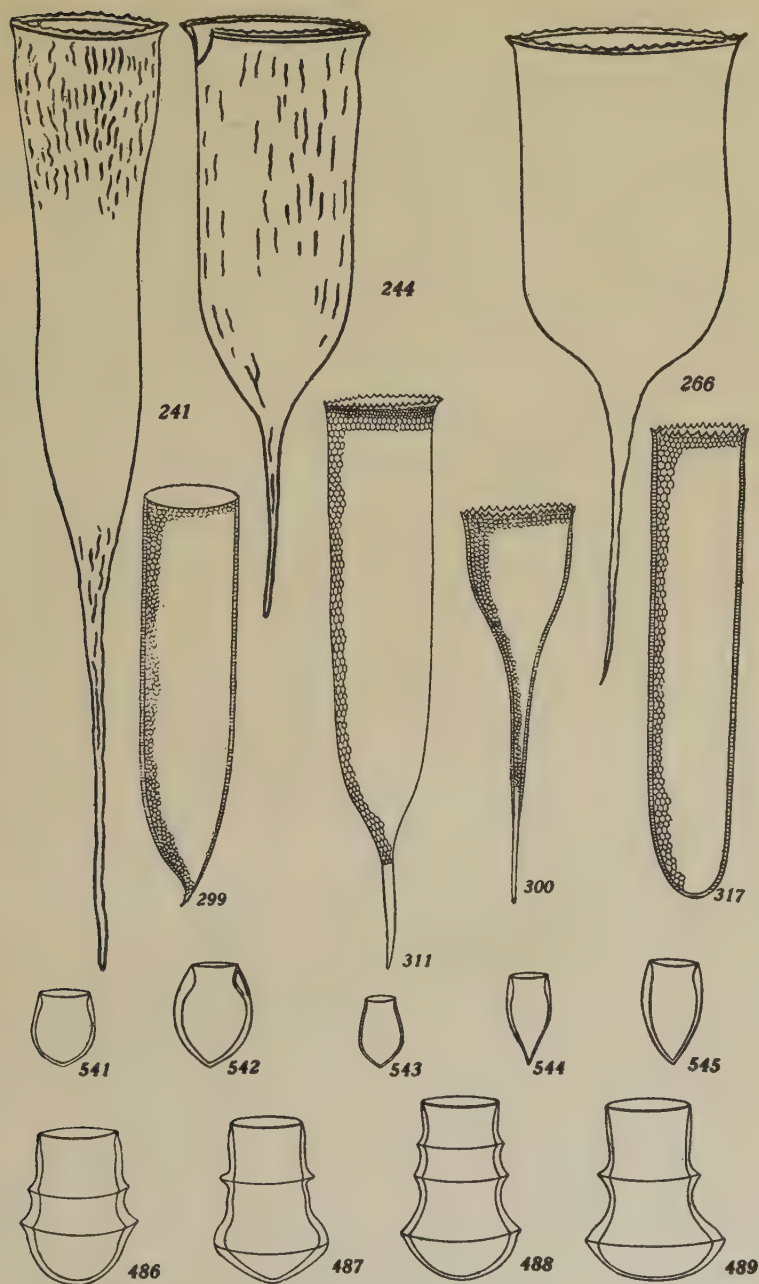


FIG. 26.—Comparison of loricae of genera from polar and from tropical seas. *Cymatocylis*, 241, 244, 266, from the Antarctic, and *Parafavella*, 299, 300, 311, 317, from northern seas. *Proplectella*, 541–545, from the tropical Pacific; and *Amplectella*, 486–489, also from the tropical Pacific. $\times 200$.

tunities for mutant changes in the lorica-forming substance and in the pattern of the genes controlling differentiation. The observed facts of speciation approximate the demands of the hypothesis.

A second instance of a more general correlation between the structure of the lorica and environmental conditions occurs in the genera restricted to neritic areas. The pelagic Tintinninea more or less invade and permeate the neritic zone, as the result of the tidal and other currents, but the neritic fauna does not to an equal degree invade the high seas. As a result of this contrast certain species, and to a less extent certain genera, have a predominating neritic habitat. This is especially true of the species of *Tintinnopsis* (Fig. 12). The neritic Tintinninea are characterized by more or less irregularity in the outer surface of the lorica, due to the agglomeration of blobs of substance resembling that of the lorica in its finer alveolar structure. These are adherent with varying regard for the general symmetry or pattern of the lorica, in most cases. In some instances foreign particles from the circumambient silt are included in the agglomerations. The loricae of species from the high seas such as those of *Proplectella* (Fig. 13) are remarkably and uniformly free from such agglomerations, and there is a noticeable contrast between this adherence to the symmetry of the pattern in loricae from the high seas and the looseness of such conformity in those of the inhabitants of the more disturbed neritic area.

The problem as to the existence in biological phenomena of anything like quanta has been raised by the work of Przibram (1929), Sztern (1914), and Koltzoff (1928). They note the duplication of genes, the doubling of gene chains, the doubling of chromosomes, and the step-like doubling of volume at moult in some insects.

It has occurred to me that the process of speciation likewise offers a field for an analogous stepping up in volume. As one inspects the assembled species of the various genera of the Tintinninea, especially those in which elongation prevails, one is very often struck by the recurrence in different genera of groups of species in each, of one general size, and of several such groups within the genus. There are also the well-known phenomena of nanism and gigantism, seemingly correlated elsewhere in some instances at least with haploid and polyploid states of the nucleus.

To test this hypothesis an attempt was made to group the 29 species of *Tintinnus* (Figs. 26 and 27) according to their computed volumes. This resulted in the emergence of 7 groups of 2, 5, 5, 5, 4, 4, and 4 species each. The original figures had been selected for the recently published conspectus (Kofoid and Campbell, 1929) without reference to this problem, but as typical of the species, among other features, in size. They therefore are not widely divergent from the modes of the species represented. In computing the step-up from one group to the next the assumption is made that the volume of the lorica would be doubled at each step.

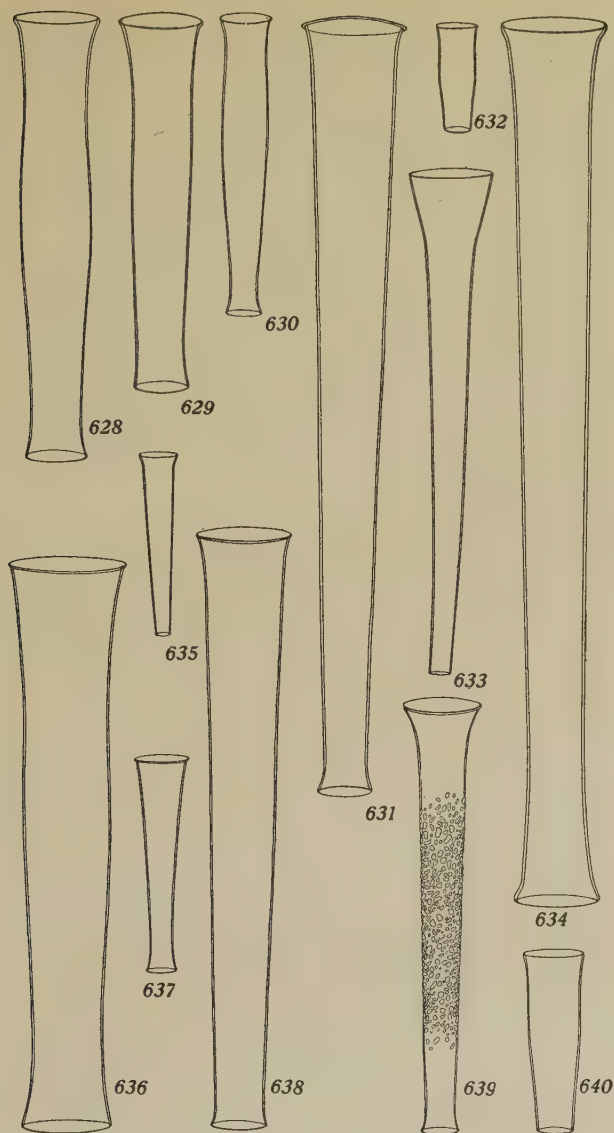


FIG. 27.—Loricae of species of *Tintinnus*. 628–640. $\times 200$.

Starting with the smallest group and stepping up the seven to the largest, we find the computed mean volumes of the loricae of the species of the several groups depart from the expected ones by +14.9, -3.6, +20, -8.3, ± 0 , and -26 per cent, respectively, and that the average departure is ± 11 per cent.

There are naturally other factors modifying the volume of the loricae, such as the temperatures at the time of secretion and formation, the volume of the cytoplasm as affected by prior and current metabolism, the unknown factors which may modify behavior during the formation of the lorica, and the rather fundamental question as to the validity of including the void within the lorica in the computed volume. In rebuttal to the suggestion that such variables invalidate any conclusion that quanta phenomena, or a biological category of quanta, can be detected in the phenomena of speciation, one can only cite the facts that within certain limits size is a species character, that inspection shows the prevalence of size groups in many genera, and that the divergence above noted in the genus *Tintinnus* from the expected is within the order of magnitude of variation in other biological phenomena. On the assumption that the genus is monophyletic, the observed facts as to volume of the lorica in the groups of species of *Tintinnus* at least suggest the quanta aspect of speciation, but still leave much room for criticism.

Investigators in systematic zoölogy and paleontologists, both of whom view the results of the process of speciation in the groups under their inspection *in toto*, or at least in so far as their evidence affords such an opportunity, have often noted what they designate as orthogenesis. Concretely this is the phenomenon of seriation of the species in one or more lines from simple to complex based on structural modifications in definite directions.

Evidences of such structural progression are abundant in the Tintinnoinea (Figs. 29–31). It is possible in the genera with numerous species to find one or usually more lines of progressive differentiation among the species. These lines are characterized by (1) increase in size, (2) duplication or multiplication of structural features such as rings, spiral turns, striae, pedicels, knobs, (3) elongation, and (4) differentiation of surface pattern. The paleontologist has in addition to the structural evidence the chronological succession of his material based on stratigraphic data, which give to orthogenesis its sequential phase. The systematist, on the other hand, lacks all chronological data and does not find much support from genetics to justify the inference that degrees of morphological resemblance are evidences of corresponding degrees of genetic relationships. In the sub-order Tintinnoinea the only approach to the time factor in the evolution of the group is to be found in the very large body of evidence that evolution has progressed most rapidly, as shown in multiplication of species and diversification of genera, in the tropical seas. Here the operation of Van 't Hoff's Law in accelerating life processes has so increased the speed of living that more evolution has happened here than in the colder polar regions within the same period.

In Figures 29–31 the simplest and the most highly specialized species

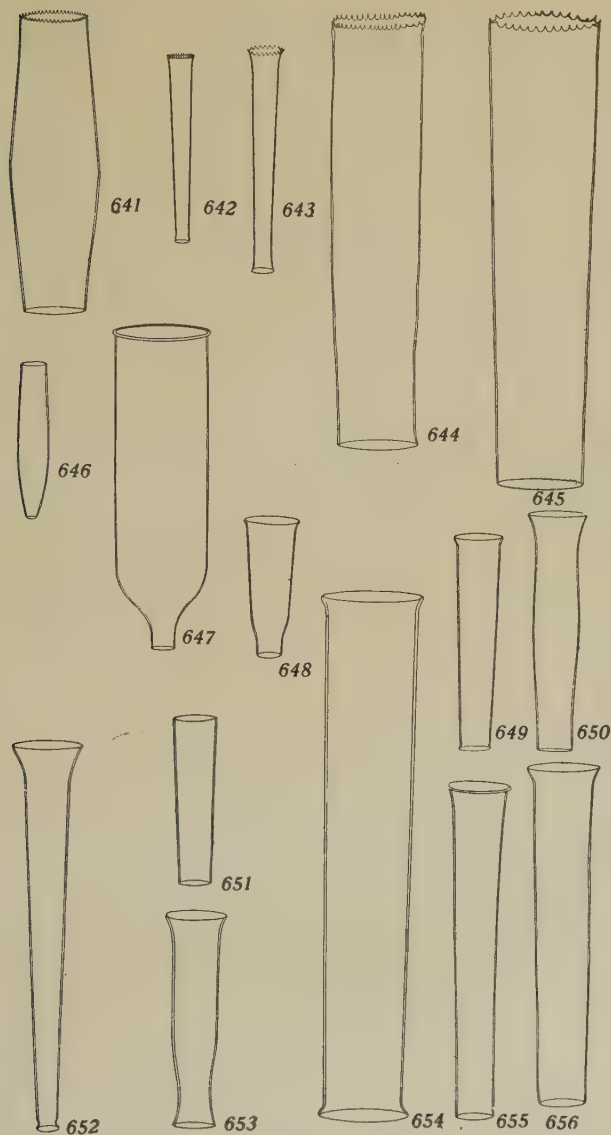


FIG. 28.—Loricae of the species of *Tintinnus*. 641–656. $\times 200$.

in each of 46 genera of the Tintinnoina are represented in pairs. These paired figures represent the ends of the single or of the main series of species if there is more than one series, in each of the genera. They exhibit the beginnings and ends of one or more of the types of structural progressions enumerated above. In some of the genera, such for example

as *Codonella* (Fig. 11), *Tintinnopsis* (Fig. 12), *Codonellopsis* (Fig. 22), it is possible on inspection to note several lines of progressive differentiation within which most, if not all, of the species may be grouped in series of species of increasing size and complexity.

In scanning the environmental horizon for some potent agency capable of inciting genetic change in the Tintinnoinea the one outstanding factor subject to extreme and repeated change in the pelagic environment is solar radiation. The diurnal and seasonal changes in the angle of incidence and in the intensity of the sun's rays, on the one hand, and the vertical selective absorption of the spectrum and its adjacent regions of the octaves of electromagnetic disturbances by the sea water, on the other hand, present both a changing and also a diversified and potent factor above the light floor in the marine environment.

The greatly expanded and progressively diminished spectrum is thus flashed daily through the light zone, with the ultra-violet penetrating most deeply and the infra-red more limited to the surface. A selective action of the light is thus available and repeated shocks are provided by the diurnal and seasonal rhythms and by vertical movement, passive or active, on the part of the organisms. The possibility of the action of other sections than that of visible light as genetic excitants is not excluded, though evidence is lacking in the sea.

FIGS. 29-31 (pp. 35-37).—Orthogenesis as shown in the loricae of the simplest and most highly modified species of 45 of the genera of the Tintinnoinea. $\times 125$.

FIG. 29.—4 and 8, *Tintinnidium semiciliatum* and *Tdm. neapolitanum*; 9 and 10, *Leptotintinnus neriticus* and *L. simplex*; 15 and 77, *Tintinnopsis nana* and *Tps. urniger*; 99 and 125, *Codonella brevicollis* and *C. cistellula*; 137 and 140, *Stenosemella inflata* and *S. expansa*; 144 and 180, *Codonellopsis tuberculata* and *C. longa*; 187 and 188, *Climacocylis scalaroides* and *C. decipiens*; 190 and 203, *Coxiella pelagica* and *C. decipiens*; 206 and 210, *Helicostomella longa* and *H. kilensis*; 211 and 222, *Cyttarocylis euecryphalus* and *C. magna*; 223 and 226, *Poroecus apicatus* and *P. annulatus*; 235 and 262, *Cymatocylis antarctica* and *C. drygalskii*; 284 and 290, *Favella azorica* and *F. brevis*.

FIG. 30.—304 and 314, *Parafavella greenlandica* and *P. ventricosa*; 321 and 332, *Epiplocylis healdi* and *E. acuminata*; 348 and 355, *Ptychocylis cylindrica* and *P. urnula*; 358 and 364, *Acanthostomella minutissima* and *A. lata*; 366 and 370, *Craterella torulata* and *C. acuta*; 372 and 382, *Metacylis lucasensis* and *M. rossica*; 383 and 390, *Petalotricha entzi* and *P. foli*; 392 and 395, *Protorhabdonella striatura* and *P. simplex*; 398 and 418, *Rhabdonella amor* and *R. conica*; 419 and 421, *Rhabdonellopsis longicaulis* and *R. composita*; 425 and 446, *Parundella minor* and *P. attenuata*; 449 and 454, *Xystonella lanceolata* and *X. scandens*; 463 and 475, *Xystonellopsis ornata* and *X. pinnata*; 486 and 492, *Amplectella insignis* and *A. quadricollaria*; 493 and 494, *Amplectellopsis angularis* and *A. biedermaani*; 496 and 497, *Cricundella tridivisa* and *C. quadridivisa*; 503 and 512, *Undella clevei* and *U. bulla*; 513 and 523, *Undellopsis pacifica* and *U. umbilicata*; 524 and 529, *Proplectella perpusilla* and *P. ovata*.



FIG. 29

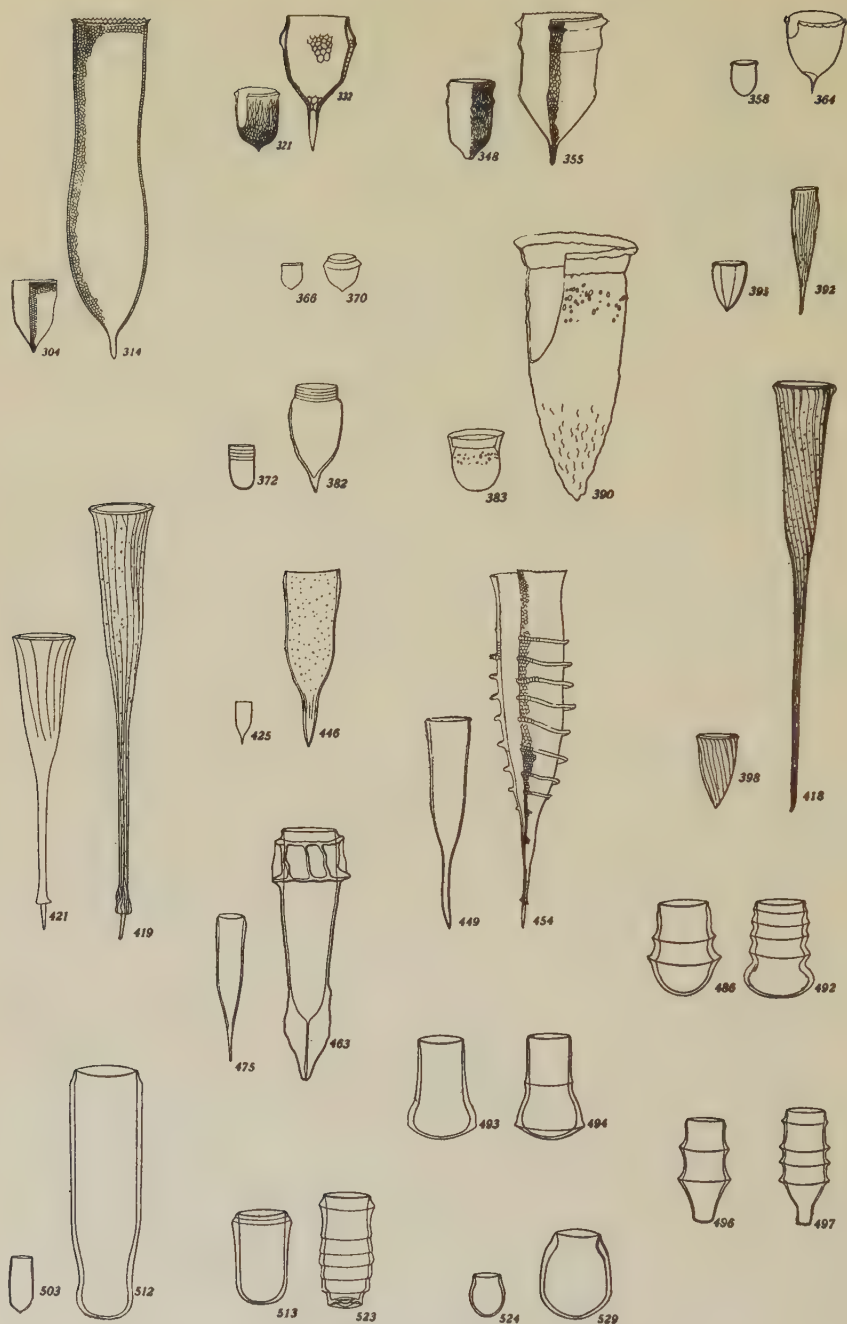


FIG. 30



FIG. 31.—547 and 568, *Dictyocysta obtusa* and *D. apiculata*; 576 and 581, *Bursaopsis vitrea* and *B. punctostriata*; 582 and 584, *Canthariella septinaria* and *C. brevis*; 590 and 592, *Amphorella minor* and *A. calida*; 593 and 597, *Steenstrupiella entzi* and *S. gracilis*; 601 and 604, *Amphorellopsis tropica* and *A. tetragona*; 607 and 608, *Albatrossiella filigera* and *A. minutissima*; 609 and 613, *Dadayiella acuta* and *D. jørgenseni*; 616 and 622, *Ormosella bresslaui* and *O. schmidtii*; 624 and 627, *Stelidiella phialia* and *S. stelidium*; 634 and 651, *Tintinnus birictus* and *T. tubulosus*; 657 and 662, *Daturella angusta* and *D. magna*; 666 and 673, *Salpingella octogenata* and *S. acuminatoides*; 691 and 695, *Salpingacantha simplex* and *S. undata*.

The differences between the belt lying betwixt the Tropic of Cancer and the Tropic of Capricorn, on the one hand, and the polar seas within the Arctic and Antarctic circles, on the other, in the matter of their relation to light, and the access of and the spread of the parts of the spectrum therein, result in marked differences in temperature and irradiation in the two areas.

How far they control and incite increase in the number of vertebrae in fishes in the Arctic and speed up the process of speciation and structural diversification in the Tintinninea in equatorial waters is at present only a matter of observation and inference. If this discursive discussion which my contact with this group has occasioned should invite experiment, its presentation will be justified.

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II. THE OCEANOGRAPHIC POINT OF VIEW

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The subject for this address was selected because several recent occurrences indicate that some who might be expected to know what the oceanographic point of view is do not know; and other occurrences indicate that one of the scientific needs of this country is for a larger number of oceanographically minded investigators. The number of broadly trained oceanographers in the United States is pitifully small, and there is a correspondingly great need for the training of such investigators. This training should be based on a foundation of the fundamental sciences, and it should consist of a general knowledge of the entire field of oceanography with intense specialization in some one field. Extensive knowledge alone is not adequate; but the results of specialized researches can be interpreted only in the light of a comprehensive grasp of the entire field of which they form a part. It is this grasp of the entire field of oceanography and the capacity to see interrelations and correlations of special phenomena with other marine phenomena that I mean by the oceanographic point of view. The sea and the interpretation of its phenomena must be the major purpose.

At first I had intended to present three illustrations of what I mean: (1) my own work on corals and the results of later researches by several others; (2) the calcium carbonate problem in the sea; and (3) the vertical and horizontal distribution of oxygen in the sea with reference to marine organisms and marine bottom deposits. It quickly became obvious that the three topics mentioned could not be adequately treated in one lecture. It was therefore decided to pay only brief attention to the last two topics, one of which is presented in detail by Dr. Moberg, to add a note on the relations of phytoplankton to chemical conditions in the sea and upwelling off the coast of southern California, and to discuss the ecology of corals more fully.

CALCIUM CARBONATE DEPOSITS IN THE OCEAN

Table I shows the relative importance of calcium carbonate in modern deep-sea deposits.¹ The range in percentage is from 4.01 per cent in Radiolarian ooze to 79.25 per cent in Pteropod ooze. In some of the shoal-water deposits of the tropics the percentage is known to be nearly as high as 97 per cent, while in some marine oölites the percentage exceeds 99 per cent. Virtually all the calcium carbonate deposited in the sea is due

¹ Vaughan, 1924, p. 313.

to chemical precipitation, which may have occurred in contact with organic tissue or which may have been due to physico-chemical conditions produced in the water by organic or inorganic agencies. The organic deposits are represented by the calcareous tests of organisms, among which pelagic foraminifera are the most important, and the inorganic deposits by marine oölite and aragonite needles.

TABLE I
PERCENTAGE OF CaCO_3 IN MODERN DEEP-SEA SEDIMENTS
(Data taken from "Challenger" report)

Area of the earth: 196,470,700 square miles

1 Name of Deposit	2 Area in Square Miles	3 Percentage of Area of the Earth	4 Percentage of CaCO_3	5 Products of Figures in Columns 3 and 4
Red clay	51,500,000	26.2	6.70*	175.54
Radiolarian ooze	2,290,000	1.1	4.01	4.41
Diatom ooze	10,880,000	5.5	22.96	126.28
Globigerina ooze	49,520,000	25.2	64.47	1624.64
Pteropod ooze	400,000	0.2	79.25	15.85
Limy mud and sand.....	2,556,800	1.3	85.53	111.89
Volcanic mud }	600,000	0.3	20.49	29.64
Volcanic sand }			28.79	
Green mud }	850,000	0.4	25.79	37.65
Green sand }			49.78	
Red mud	100,000	0.05	32.28	1.61
Blue mud	14,500,000	7.3	12.48	91.10
		67.55		2175.27

$$\frac{2175.27}{67.55} = 32.20 \text{ per cent}$$

$$\text{CaCO}_3 = 32.20 \text{ per cent; CaO} = 32.20 \times \frac{56}{100} = 18.03 \text{ per cent}$$

Concentration of CaO in deep-sea sediments $= \frac{18.03}{5.08} = 3.55$ times the percentage in average for the earth's crust.

* Mr. George Steiger informs me orally that the CaCO_3 in red clay is about 9 per cent.

In the sea there is gradation from conditions of precipitation to conditions of solution. In order to solve this problem, or set of problems, several things need to be known. The first is, what are the conditions, and what in sea water controls the conditions under which precipitation, preservation, or solution may take place? Those who are now studying the problem find

that some of the opinions formed thirteen or fourteen years ago need revision. Because sea water is a very complex salt solution, results obtained from experiments on the solubility of calcium carbonate in simpler solutions are not entirely applicable to sea water. The handling of the problem according to the principles of physical chemistry has defied those who have made such attempts, just as the problems of the blood have. At present it seems that empirical results from controlled laboratory experiments offer the most hope for advance, and such experiments are now being conducted by Moberg and Gee at the Scripps Institution. The experiments, however, cannot be applied to the sea unless the investigator knows the material whose origin he is trying to explain and the conditions under which that material occurs in the sea. In the shoal warm waters of the tropics calcium carbonate is accumulating and there it is preserved; but in or above the deep-sea Red clay and Radiolarian ooze areas it has been or is being dissolved. The action of the sea in each of these areas is the reverse of what it is in the other. We need to know the reasons for such conditions, not merely qualitatively but quantitatively.

OXYGEN IN THE ATLANTIC AND IN THE EASTERN PACIFIC

Since the subject of oxygen in the Atlantic and the Pacific has been discussed comprehensively by Dr. Moberg only a brief treatment is needed in this connection. He has prepared for me the following note:

In all localities the first 100 meters below the surface are roughly saturated with oxygen, the quantities varying with temperature from about 4.5 to 8 cc. per liter.

Below 100 meters the quantity decreases until in most localities a minimum is reached at from 400 to 1,000 meters. The amount of oxygen in the minimum layer varies with latitude and other factors. In the Pacific it appears to be considerably lower than in corresponding latitudes in the Atlantic; for example, between latitudes 20° and 30° N. in the eastern Pacific it may be as low as 0.2 cc. per liter, or 1 cc. per liter at the most. At the corresponding latitude in the Atlantic the minimum quantity is from 2.5 to 4 cc. per liter.

Below the minimum layer the oxygen rapidly increases in the Atlantic to 5 cc. or more, whereas in the Pacific the quantity in the region investigated never reaches 4 cc. per liter. Consequently it appears that at most depths the oxygen in the Atlantic is from 1 to 3 cc. per liter higher than in the Pacific.

The possible significance of the vertical distribution and the difference in the vertical distribution of oxygen in the ocean is manifold. First, the possible relations to organisms will be mentioned; the relations to corals are considered near the end of this address.

One of the researches under way at the Scripps Institution is a study of the oxygen consumption of certain fishes, especially the killifish (*Fundulus*) and the green fish (*Girella nigricans*) by Keys and Wells. Keys, in a paper recently published, "Influence of Varying Oxygen Tensions

upon the Rate of Oxygen Consumption in Fishes," gives the O_2 consumption of *Fundulus parvipinnis* as between 0.13 and 0.23 cc. per hour per gram at $20^\circ C$. when the O_2 tension ranges from 4.64 to 5.26 cc. per liter. His conclusions are as follows:

(1) The oxygen consumption of fishes is relatively independent of small deviations from the normal oxygen tension of the medium, (2) the respiration of fishes is very markedly decreased at or near the asphyxial level of oxygen tension, and (3) it should be quite possible, by employing modifications of methods previously used, to determine something in the nature of a standard metabolism of fishes.

How does the oxygen-tension needed for the life of *Fundulus* compare with oxygen-tension at different depths and different places in the ocean? In the Atlantic the O_2 below 1,000 m. is about 5 cc. per liter. What influence lower temperature and lower tension may have on the availability of the oxygen is not certainly known, but the quantity would seem to be adequate, and this suggestion is corroborated by the abundance of deep-sea fishes in the Atlantic. But in the Pacific is the quantity always above the asphyxial level? Is knowledge of asphyxial levels for different species of fish at different temperatures and tensions sufficient to warrant an opinion? Apparently there is sufficient oxygen to support fishes in a quiescent state, but what would be the effect of the low tensions if they should become active? What are the oxygen requirements and asphyxial levels of the invertebrates? Something, but not enough, is known of this subject. A series of researches of significance as to the relations of marine organisms to their environments has been formulated.

A few words about marine bottom deposits: Red clay in the Atlantic Ocean has its minimum depth at only slightly less than 3,000 fathoms, whereas in the eastern Pacific it is found at a depth of 1,955 fathoms and its average depth there, according to Murray and Lee, is 2,404 fathoms. Red clay contains but little calcium carbonate, average about 9 per cent, although organisms with calcareous tests may be abundant in the surface waters. The lower oxygen content of the deeper water in the eastern Pacific is coincident with reduced pH values and it appears that it may be correlated with a greater capacity of the water to dissolve calcium carbonate and may therefore be correlated with the presence of Red clay at intermediate depths in the eastern Pacific. Attention should be called to an apparent paradox, which is that Red clay represents the most highly oxidized marine sediment. It is probable that the oxidation took place before the material reached the bottom.

For the handling of problems of the kind outlined, physiologists, chemists, and students of bottom deposits are needed, but only one who has a wide knowledge of oceanography can discern the probable interrelations between the complex phenomena.

PHYTOPLANKTON, CHEMICAL CONDITIONS IN THE SEA, AND UPWELLING

Before proceeding to an account of the ecology of corals I shall call attention to an article by Dr. Moberg entitled "The Interrelation between Diatoms, Their Chemical Environment, and Upwelling Water in the Sea, off the Coast of Southern California." In this paper he shows that between depths of 30 and 50 meters there is the maximum abundance of diatoms; and above those depths there is decrease in the quantities of PO_4 , NO_3 , and CO_2 , and increase in pH, intensity of light, and temperature. The SiO_2 decreases in quantity toward the surface, but it is peculiar in that there is no significant change in the trend of its amount above 50 meters. The O_2 relations are not given. Conditions off the west coast of Africa, according to Wattenberg, are virtually identical. The study and interpretation of phenomena of the kind presented require knowledge of planktonic organisms, the physical and chemical properties of the sea water, and the circulation of the water. A rough translation of two sentences from Wattenberg is as follows:

Chemical reactions constitute the meeting place between the work of the chemist and that of the biologist, geologist, and mineralogist: respiration, assimilation, the nutrition of plants and animals on the one hand, the formation and the changes of the sediments, in so far as they are chemically conditioned, on the other, are here the most important processes.

Hence, it is clear that the co-operation of chemists, oceanographers, biologists, geologists, and mineralogists is necessary in the study of the material in order to evaluate the results of each individual discipline.

ECOLOGY OF CORALS

When about twenty years old I decided to study geology with the intention of trying to reconstruct the conditions under which marine deposits were laid down in the sea. My first large work was a monograph of the Eocene and lower Oligocene coral faunas of the United States. Besides describing the faunas and using them for the characterization of the different geologic formations, I compiled all the information that I could get on the conditions under which the most nearly related forms now live in the sea, and from such data attempted to infer the conditions, especially depths, under which the corals of each horizon lived. The attempt was to revivify the seas of the past. This work was published in 1900.

My next large work was a monograph of the corals of the Hawaiian Islands and Laysan. One who casually looks at this book and its 96 plates may think, erroneously, that its primary purpose was a systematic description of the coral faunas of that part of the world. The Hawaiian collections extended from very shoal water to a depth of about 1,000 fathoms and they furnished an opportunity to study the different kinds of corals with reference to depth, temperature of the water, and kind of bottom. The purpose of the investigation was to get all the information possible on the

ecologic relations of the different kinds of corals, with the intention of utilizing it in interpreting the seas of the past. The major results of this investigation were summarized in tables of numerical distribution of Hawaiian corals according to depth; the bathymetric distribution of the genera; and the thermal distribution of the genera.

In acceptance of an invitation from Dr. A. G. Mayor, I began in 1908 field and experimental studies of the corals in Florida and the Bahamas and prosecuted the investigations in that region for eight consecutive years. The initial purpose of the investigations was to find out the relations of corals to their environment and to ascertain the rôle of corals as rock builders. The program of study comprised the relations of corals to (1) nature of the movement of the water; (2) depth; (3) light; (4) temperature; (5) salinity; (6) sediment; (7) exposure to the air; and (8) food. In addition to the subjects mentioned, rearing coral planulae and an extensive series of experiments on growth-rate were undertaken. Although it is now an old story, I shall quickly review the more important results of the investigations.

Relations of corals to the nature of the movement of the water.—In shoal waters those corals that are subjected to the beat of waves and surf must have strong skeletons to withstand the buffeting, while those that live in the quiet waters of lagoons or at depths below the level of violent agitation may have fragile skeletons. Some massive species may be represented in both environments. Other species that are present in both exposed and protected situations are to some degree dimorphic; that is, the colonies in exposed situations produce compact coralla, while those in quiet water produce colonies with relatively slender, fragile branches. It is possible to change the compact growth form of a colony to a form with slender branches by transferring it from rough to quiet water, as was shown by experiment.

Depth relation of corals.—The general relations of corals to depth were as was found for the Hawaiian Islands. Vigorous growth of reef corals is usually in water not over 100 feet deep, although some shallow-water species extend into deeper water. Depth is concomitant with light and temperature, and, in places, also with sediment.

Relations to light.—All the observations and experiments that I made on reef corals with reference to light indicate that rather strong light is essential for vigorous growth. The observations of natural conditions showed no reef corals in dark places and specimens of 17 species placed in a live-car from which light was excluded either died or showed the effects of the unfavorable environment. The light problem in reef corals is intimately bound up with the activity of the symbiotic zoöxanthellae and it will be further discussed in connection with the account of the food for corals.

The depth of penetration of the different wave-lengths of light into sea water has not been satisfactorily worked out. The best results are probably those obtained by Helland-Hansen by the use of a photometer he designed. South and west of the Azores he found at a depth of 100 meters that red rays were weak, but blue and ultra-violet were stronger. The blue attained a depth of 500 meters and the ultra-violet a depth of 1,000 meters, but no light reached so deep as 1,700 meters. The most abundant deep-sea corals, in depths between 180 and 500 meters, therefore, are reached by blue and ultra-violet but not by red light. The deepest deep-sea corals live in perpetual darkness and at temperatures between 1° and 4°C.

Discussion of the penetration of light into sea water cannot be introduced here, but it may be said that more information on the subject is urgently needed.

Relations to temperature.—The experiments to determine the temperature ranges that shoal-water corals can endure were conducted by Mayer. Except on very shallow flats and in pools, where the water may be stagnant at times and become heated to a temperature between 33° and 38°C., the upper limit of the temperature endurance of corals is rarely or never reached. Therefore, attention will be paid only to the lower limit. According to Mayer, if the temperature of the Florida reef tract were lowered to 13.9°C. all the principal reef corals would be killed, but the principal inner flat species would survive. Mayer got similar results at Murray Island, Australia.

Temperature records for the Florida reef indicate that the corals do not naturally withstand so much cooling. An extensive set of records for the Florida reef, supplied by the Bureau of Fisheries, indicate 18.5°C. as the minimum temperature that a vigorous reef will endure, and it is probable that it can withstand such a temperature for only a short time. From a study of records of reef areas I have expressed the opinion that the annual minimum temperature must not be below 18°C. and the average for the coldest month in the year not below 22°C. The known temperature gradients in coral reef areas are not sufficient to inhibit the life of reef corals at the maximum depth to which they extend. The limitation in depth is, therefore, due to other factors.

Relations of corals to salinity.—Under ordinary conditions in the sea the limits of salinity range that corals will endure are not passed. They will live in water that contains between 27 and 39 parts per thousand of sea-salts. Apparently they will not live in concentration of salts much beyond the maximum concentration in the sea. Near the shore corals may be killed by freshets from the land, and reefs exposed at low tide may be damaged by heavy rains.

Relations to sediment.—All corals that I studied possess some capacity

for ridding their surfaces of sediment, but this capacity is not the same for all corals. The massive forms on the exposed reefs in general possess the least ability to remove sediment dropped on them. They depend on the agitation of the water for keeping their surfaces clean. The species that live on the bottom of the lagoons and on the inner flats are the most competent in removing sediment. The sediment is removed by becoming imbedded in mucus and then being wafted by the cilia to the periphery of the colony, where it is dropped off the edges. Many species that live where there is sediment are branching forms, on which sediment cannot readily find a resting-place.

Mayer thought that he found a correlation between the ability of corals to withstand burial beneath sediment and ability to endure high temperature. Whether or no there is any physiologic basis for such a correlation, the species of corals that live on the inner flats and in pools are the ones that can best endure sediment, rid their surfaces of sediment, and withstand the greatest temperature ranges.

Relations to exposure to the air.—Experiments were made on sixteen species of Tortugas corals. All of them endured exposure on a glass plate in the shade for half an hour, nearly all endured an hour's exposure, and some survived four hours' exposure. Colonies of some species withstood exposure for one and a half hours on a glass plate in the sun. In general the ability to withstand exposure to the air is a function of the porosity of the skeleton. The inner flat corals are more resistant than the species from the exposed reefs. It is this capacity to endure exposure to the air that enables corals to grow above extreme low-tide level. Mayer's experiments at Murray Island gave results similar to mine for Florida.

Food.—The most elaborate laboratory experiments that I made on corals were on their mechanism for catching food and for cleaning their surfaces of sediment, and on the nature of their food. It is generally known that the entire ectoderm of corals contains nematocysts which can shoot their darts into prey and hold it if it is not too strong. The ectodermal surface is ciliated and secretes mucus, in which nutrient or non-nutrient particles may become imbedded. The cilia in response to certain stimuli beat toward the mouth, in response to others they beat from it, and particles imbedded in the mucus are moved accordingly. Ciliary reversal occurs. The tentacles have adhesive tips and are effective in catching food. The mesenterial filaments may be extruded through the column walls and the mouth and they both catch and digest food outside the gastric cavity.

A large variety of food was offered the corals. They took any kind of animal food, such as meat juice, crab meat, pieces of fish, and every kind of zoöplankton offered them; but they would not eat any kind of purely vegetable food. They were offered diatom mat and chopped-up seaweed

but refused such food unless it was coated with animal matter; then it would be swallowed and later the vegetable material would be ejected. An experiment tried several times was to place on one side of a polyp mouth a piece of diatom mat and on the other side a piece of crab meat. Invariably the crab meat was swallowed and the diatom mat was not. Copepods were dropped by means of a pipette within the tentacles, which acted with lightning-like rapidity, and none was ever seen to escape.

The results of one significant experiment that I conducted have not been published, and, because of their pertinence, they will be given here. On July 5, 1912, a specimen of *Maeandra areolata* was placed in filtered sea water and kept in such water for five days; that is, it was starved for five days. At the end of the period of starvation, its tissues had become thin, emaciated, and pale. The same specimen was then placed in the ordinary sea water of the locality, Loggerhead Key, Tortugas, Florida, and artificially fed with animal food. Ten days of such feeding were required to restore the colony to the condition that it was in at the beginning of the starvation experiment. If zoöxanthellae are of so much value as food, why did the coral so quickly show the effects of being deprived of animal food and why was so much animal food required to restore it to its condition prior to its starvation? The answer appears to be obvious.

These experiments led to the conclusion that the food of corals consists solely of animal matter. Several have contended that the symbiotic zoöxanthellae constitute a source of food for corals. I have combatted this view because of the experiments that I had conducted. The results obtained by subsequent investigations will be presented later.

The depth range of reef corals may be limited to the photosynthetic zone of green plants because of their need of the oxygenating influence of the zoöxanthellae. The vertical distribution of zoöplankton may also be a limiting factor. But information on this subject is still inadequate for trustworthy conclusions.

Rearing coral planulae.—Coral colonies were brought into the laboratory and any planulae that were extruded were removed by means of a pipette to jars, on the bottoms of which were terra-cotta disks. Care must be taken in rearing planulae to the attachment stage. Clean water is necessary, and precautions are needed to prevent their escape. The duration of the free-swimming stage ranged from 2 to 23 days. The possibility of wide distribution of many species by means of surface currents is obvious.

The disks to which the planulae attached themselves were planted in the sea on the heads of iron stakes. Forty-three colonies lived to be one year old, and thirteen attained an age of five years before they were taken up.

Growth-rate of corals.—The growth-rate of corals was investigated by measuring (1) colonies reared from planulae; (2) corals cemented to disks of terra-cotta or cement and attached to the heads of iron stakes in a variety of situations both in Tortugas, Florida, and off Andros Island, Bahamas; and (3) colonies living naturally in many places. The corals in Florida were measured annually, those in the Bahamas two years after the first measurements. The growth-rate of 25 species was investigated, and a total of some thousands of measurements were made.

The size of the colonies of all species of corals seems limited, but some attain large dimensions, 2 to 3 meters or even more in diameter, and nearly as much in height, while other species are adult when a diameter of 35 to 50 millimeters has been reached.

The upward growth-rate of *Orbicella annularis*, the principal reef builder of the Pleistocene and living reefs in Florida and the West Indies, is from 5 to 7 millimeters per year, according to station. At 6 millimeters per year, it would form a reef 150 feet (= 46 meters) thick in 7,620 years; at 7 millimeters per year it would build the same thickness of rock in 6,531 years. *Acropora palmata*, which grows more rapidly, might build a similar thickness in 1,800 years.

Growth-rate is one of the important factors in the battle between corals and some of their natural enemies. For instance, if corals grow less rapidly than sediment is being deposited on the bottom, although other conditions may be favorable for their life, they will surely be killed by smothering. In the competition between attached and incrusting organisms, growth-rate is one of the most important factors in determining which shall survive. Among the inimical organisms are various marine algae, including the calcareous *Halimeda* and incrusting nullipores; other such organisms are sponges, tunicates, Bryozoa, and pelecypods.

A study of the growth-rate of corals has an interest not only in understanding the rate at which they form rock, but also in understanding their struggle for life against enemies, both organic and inorganic.

SUMMARY OF STATEMENTS ON REEF CORALS

The conditions necessary for the vigorous growth of reef-forming corals are: (a) Depth of water, maximum, about 45 meters (25 fathoms); (b) bottom firm or rocky, without silty deposits; (c) water circulating, at times strongly agitated; (d) an abundant supply of small animal plankton; (e) strong light; (f) temperature—annual minimum not below 18°C., minimum average temperature for the coldest month in the year not lower than about 22°C.; (g) salinity between about 27 and about 38 parts per thousand.

According to conservative estimates, reef corals can build a reef 46 meters (150 feet) thick within a period ranging from 1,800 years to 7,500

years; but, in places, a reef of such a thickness might be formed within 1,000 years, according to Gardiner.

RESULTS OF INVESTIGATIONS BY OTHERS

So far I have presented the results almost exclusively of my personal work. I was the first to conduct such a series of field and laboratory investigations, but similar methods were soon taken up by others and important results were obtained. Mayor in 1913 led an expedition to Murray Island, Australia, and later he spent several seasons in American Samoa. I helped Mayor in studying the corals and marine bottom samples obtained in both Australia and the Samoan Islands. Mayor showed that the same ecologic principles that governed the Atlantic and Caribbean corals applied to those in the Pacific. The Pacific corals appear to grow faster than those in the Atlantic.

Later C. H. Edmondson instituted an extensive series of experiments on the general ecologic relations and the growth-rate of the Hawaiian corals. He has given us a large volume of data on the corals of that part of the Pacific. The growth-rate of the Hawaiian corals is about the same as that of the Floridian and Bahamian corals, and it seems to be less rapid than in the more equatorial part of the Pacific. Within the Pacific, corals appear to grow more rapidly near the Equator than near the margins of the tropics. The subject needs further investigation. If the indicated relations are found to be true, they will need to be explained. Seasonal variation in the temperature of the water may be a controlling factor; but possible seasonal variation in the quantity of zoöplankton in the marginal areas also needs to be considered.

Several investigators have recently studied the rôle of zoöxanthellae in the life of corals, oxygen consumption of corals, and the food of corals. The names of four of them are A. G. Mayer, H. Boschma, J. Verwey, and C. M. Yonge.

Although Boschma's experiments, conducted at the Scripps Institution, on the sea anemone, *Cribina xanthogrammica*, showed that the anemones would not grow unless fed animal food, he thought that he detected evidence of the digestion of zoöxanthellae.

The capacity of corals to digest zoöxanthellae seems to have been definitely settled by C. M. Yonge on the Great Barrier Reef. He studied the digestive enzymes of corals by means of extracts of the mesenterial filaments of *Lobophyllia* and fluid from the coelentera of a large species of *Fungia*. In the former there are a powerful protease, an extremely weak lipase, and enzymes capable of very slowly digesting starch and glycogen, but no other carbohydrate. Apparently the extract has no action on the symbiotic algae. Enzymes in the coelentera of *Fungia* are confined to protease. Experiments on *Fungia*, *Favia*, *Psammocora*, and *Galaxea*

demonstrated that fed corals continue in perfect condition in both light and dark, paling somewhat in the latter, owing to the death of the algae, but starved corals quickly begin to shrink in the tissues, undamaged algae being extruded in great numbers and the tissues consequently turning pale. This happens in both light and dark. Newly settled planulae of *Pocillopora* were placed in light and darkness, in both cases fed, and after six weeks those in the light had abundant algae, especially in the tentacles, while those in the dark, apparently just as healthy, were pure white with transparent tentacles. The only conclusion to be drawn from these results, taken in conjunction with the experiments on the feeding and digestive enzymes of corals, is that the algae are not and cannot be used as food by the corals.

Oxygen production of the zoöxanthellae has been investigated by Mayer, Verwey, and Yonge. Verwey has given a succinct summary of the results of other investigators as well as his own.

McClendon (1918) observed at Tortugas, Florida, a rise and fall in the oxygen content of the water similar to the rise and fall in the Bay of Batavia. His figures ranged from 3 to 4 cc. per liter about dawn to 4.5 to 7 cc. at 3:00 P.M. In experiments by Verwey terminal branches of *Acropora hebes*, weighing 61 g., consumed during six hours in the dark about 8.9 cc. Thus the total production of oxygen by photosynthesis in six hours amounted to about 18.2 cc. for the pieces weighing 61 g., much more (more than 30 cc.) for the pieces weighing 74 g.

From these observations it is evident that the consumption of oxygen by a coral reef must be very considerable. Assuming that a body of water in communication with the sea incloses a reef 100 meters long, 10 meters across, and 2 meters thick, and shows a lowering of the oxygen content from 5 to 3 cc. per liter, there would be a consumption of more than 4,000 liters of oxygen during a single night. If the night should last twice as long, the whole reef would suffer greatly from lack of oxygen.

Verwey, in the paper from which I have quoted, misrepresents my views regarding the interrelations between corals, zoöxanthellae, and the indirect influence of light. He actually inverted my opinion. He overlooked that I had said in discussing the relations of corals to light, "The commensal green algae, known as zoöxanthellae, that as a rule are imbedded in the tissues of shoal-water corals, set free oxygen which is intimately available for use by the corals, as it is in immediate contact with the animal tissues. Since these plants while in the dark cease to set free oxygen, and the corals under such circumstances are deprived of oxygen from that source, it may be that the poverty of coral growth in dark places is due to the suppression of the activities of these plants."¹

Mayer, and later Verwey, attempted to make estimates of the oxygen consumption of corals per kilogram of living tissue. Because of the large proportion of skeleton to soft tissue, corals are not suitable material for accurate estimates of that kind. A. B. Keys, of the staff of the Scripps

¹ Vaughan, 1919, 2d paper, p. 204.

Institution, whose paper on oxygen consumption in fishes has been mentioned, has gone into this matter with me, and it is our opinion that results of studies of the oxygen consumption of corals is of qualitative but not of quantitative significance. Information on the minimum oxygen tension necessary for the life of corals seems to be lacking. Mayor showed that seven species of Florida corals could live eleven hours in water deoxygenated with an air pump, but the actual oxygen tension is not stated. However, the opinion of Verwey that "a reef of some thousands of kilos consumes several hundred liters of oxygen during the night" may be accepted as well founded.

Verwey made a study of the effect of silt in suspension on the penetration of light, using the Secchi disk. He says that the average content of silt on the reefs he studied may be so high as materially to affect the penetration of light into the water, as at least the quantity of light corresponding to the "compensation point" of the algae seems to penetrate only about 7 to 8 meters deeper than where Secchi's disk disappears to the eye.

FINAL NOTE ON CORALS

In their general features the ecologic relations of corals as I outlined them have not been significantly changed, but some modifications probably should be made. It is very doubtful if reef corals will thrive at a depth so great as 25 fathoms (45 meters) unless the water is extraordinarily limpid; and I may have underestimated the tolerance of corals for silt.

My own work was defective on the physiological side. I have recognized this and have said that I had formulated a series of problems for attack by physiologists. We are fortunate in having enlisted the activities of such men as Verwey and Yonge, both of whom have made noteworthy contributions to our knowledge of corals. But many unsolved problems of coral physiology remain.

My purpose in undertaking researches on corals was to ascertain, if possible, the relations between corals and their natural environment. Stated in another way it was to ascertain the expression of the effects of different environments on members of a group of organisms which were to be used as indices of particular kinds of marine or oceanic conditions that prevailed in past geologic time. For the living organisms, the point of view was from the environment toward the organism; for the fossil organisms, it was from the organisms toward the environment.

Although they are not specifically considered in this lecture, there are interrelations between the marine sediments associated with bottom living organisms and the environment; and, if those interrelations are known, sediments can be utilized for inferring environmental conditions. For this reason I paid about as much attention to the marine bottom deposits as to the organisms.

CONCLUSION

Before coming to the final two paragraphs of this lecture I should like to say, as an interjection, that many who have not studied the sea apparently do not adequately realize two of its salient characteristics. They are its lack of uniformity, and the perpetual change within limits within it. There is variation from place to place and at different depths in nearly all the features of the sea; and there is also variation in time, hourly, daily, and, in most latitudes, seasonal. There is variation according to both space and time. These facts should be constantly in the mind of anyone studying the sea.

Laboratory experiments under limited conditions may yield results which are valid for those conditions, but conditions in many places in the sea may be utterly different, and because of such differences in conditions the laboratory experiments may have little or no oceanographic significance. Considerable work of this kind has been done.

In this lecture several instances have been given of what I consider the oceanographic point of view. For that point of view the dominant consideration is the ocean. The first endeavor should be to acquire as comprehensive a grasp of its varied and changing phenomena as knowledge at the time and human capacity will permit. Both knowledge and human capacity are limited. The second endeavor should be to study intensively specific phenomena and to ascertain the relations that such phenomena bear to other phenomena in the sea. Unless the results of special investigations are correlated with other marine phenomena, they should not be classified as oceanography.

What does the oceanographic point of view hold out to the biologist? It is not the strictly biologic point of view, for it looks first to the environment, the external medium, and the organisms are considered in their relations to that environment. But the validity of the Newtonian principle of action and reaction should not be forgotten. The environment affects the organisms, which react and not merely change the environment, but, through their activities, are in large measure responsible for making it what it is. May not this method of approach be the one most fruitful in the effort to understand the processes whereby organisms have achieved adjustment to the conditions under which they live?

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III. A SURVEY OF SOME OF THE METHODS USED IN MODERN DYNAMICAL OCEANOGRAPHY

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In addition to the more evident phenomena of wave motion and to relatively rapid currents occasionally noted in the sea, the water flows in a vast interconnected system of courses. This less apparent motion so characteristic of the oceans and denoted by the terms current, stream, or drift is a world phenomenon exerting a powerful influence on climate and weather as well as on the distribution of life in the sea. For centuries the subject of oceanic circulation has engaged the serious attention of practical navigators as well as geographers, geologists, meteorologists, and those engaged in other scientific pursuits. The early knowledge of ocean currents must have been of a fragmentary and general qualitative character based upon such simple evidence as "streaks" occasionally visible at current borders, the distribution of various floating objects, and the more striking differences of temperature and color.

Our knowledge of oceanic circulation cannot in general be obtained directly. Inferences must be drawn from various kinds of observations in order to build up a connected and consistent body of information about oceanic circulation. Because of the great extent of the phenomena and the relatively meager amount of pertinent observational data, varied and conflicting conclusions were reached regarding oceanic circulation long after both laboratory and theoretical physics were well established. Even at the present time we have detailed knowledge of the circulation in but few very limited regions.

A review of oceanographic literature shows that in general, even long after the earlier fantastic views were given up, each individual, proceeding from a central idea that appeared to include at least the leading factors applicable to a particular type of current or to some limited region, prematurely assumed that his conclusions from such data held for the whole system.

Before the publication in 1875 of Croll's (3)* epoch-making work, *Climate and Time*, it was deemed sufficient in oceanographic investigations to point out certain agencies that seemed to be adequate to produce the effects observed. In contrast with the practice in laboratory branches of physical science and in astronomical science, little attention was paid to proving the assumed agencies to be quantitatively adequate. The year 1884

* See numbered items in "Literature Cited" at the end of this paper.

marks the introduction of new and accurate methods of observation by that resourceful officer of our navy, Lieutenant Pillsbury (13), in his extensive and successful use of current meters in the Gulf Stream. From then on, progress in the science of physical oceanography has been rapid and productive of encouraging results.

According to the French oceanographer Thoulet (18) this science has for its object "the application to the natural phenomena of the sea the precise methods of the exact sciences, mathematics, mechanics, physics, and chemistry." When ideas founded on general impressions regarding oceanographic phenomena were examined critically from this point of view, involving numerous and accurate measurements and the application of established principles of physics, many earlier notions had to be set aside as inconsistent. However, when proceeding in this way, a thorough appreciation of all factors in the problem and an open-minded, unprejudiced attitude are requisite in order to avoid serious errors. The abstractions and simplifications necessary to the mathematical formulation of solvable problems tend to lead to merely speculative results, which, although quantitative, may be as erroneous as some of the earlier qualitative ones. Consider for example the fallacy of certain criticisms of proposed causes of ocean currents and the failure of certain abstract theories.

A French engineer, Dubuat, concluded from experiments on the flow of water in canals that a slope of 1 in 500,000 was barely adequate to produce a perceptible flow, and that all motion would cease when the slope was reduced to 1 in 1,000,000. Croll, who was a strong advocate of the wind theory of ocean currents, found that the effective slope from the Equator to the Pole, estimated from the observed differences in specific gravity, was about 1 in 2,000,000, which according to the results of Dubuat's experiments would be insufficient to generate or maintain a flow. But Dubuat's experiments were made on a small scale, and had reference to the sensible movement of water flowing over solid surfaces and retarded by its friction against them. Hence such experiments alone do not warrant the conclusion that the force due to differences in specific gravity is inadequate to cause the water of the ocean to seek its own level.

Again, a theoretical objection to the wind theory was based upon the fact that since the energy of the ocean currents exceeded that of the overlying air currents, the latter could not give rise to the ocean currents. But when we consider that the motion of the water due to winds is the summation effect of the energy of the latter exerted during a long period of time, the objection loses its force. However, it is of interest to note that if we equate the kinetic energy of a unit volume of air to that of a unit volume of water, the velocity of the water is found to be about one-twenty-eighth that of the wind. As a matter of fact this ratio is a rough average of the results of direct observation, and agrees well with the em-

pirical rule adopted by French authors that the ratio of the water velocity to the wind velocity was inversely proportional to the square root of the ratio of the density of water to that of air.

Again, in 1884, Hoffman (7), a strong advocate of the wind theory, rejected the idea of steady vertical currents in the sea. He believed that there were currents with a vertical component, but held that they were produced by "aspiration"; that is, he believed them to be the indirect effect of pressure due to the varying velocity of surface currents that produce by friction a slower motion of deep water. In support of his views he cites the following theoretical conclusion due to Kirchoff and Zöppritz as proof of the impossibility of a steady rising of deep water to replace surface water that has been removed. Assuming for simplicity a sea of uniform depth to be inclosed in a rectangular parallelepiped, Zöppritz sought to deduce the effect of a steady wind blowing over the surface from one end to the other. While an exact solution of this hydrodynamical problem could not be worked out, it was proved mathematically, that, on the assumption of steady motion, a particle not originally in the surface could not reach the surface, and a particle in the surface would remain there. But if we only stop to consider the great departure of the complex conditions in nature from the necessarily simple ones used as a basis for mathematical reasoning, it is evident that the corresponding conclusions cannot be implicitly trusted to agree with the facts of nature; and Hoffman's inference, from the application of hydrodynamical theory by other investigators, that a particular type of motion is impossible, does not settle the question.

Let us consider some of the difficulties arising from attempts to deduce laws of oceanic circulation from a simple set of assumptions and numerical data obtained in the laboratory. The German physicist, Zöppritz (20), first appreciated the importance of the viscosity or internal friction of water, in investigations on ocean currents and in 1878 he published the first mathematical theory of wind-driven currents. Before this the opinion prevailed that a difference of level was necessary for the production of a current. It was still believed, as stated by Varenus in the seventeenth century, that "water has no natural motion except that which follows when it flows to lower levels." But Zöppritz (20) concluded, from the theory of a viscous fluid and the coefficient of viscosity determined in the laboratory, that the wind could produce a surface current by its friction on the surface layer which would in turn move the water underneath, and thus in the course of time could give rise to motion throughout the whole body of water from the surface to the bottom. In agreement with this, the idea that the wind can produce a current by the horizontal force of friction on the surface has always been held by practical seamen. They have always regarded the wind as the main cause of all currents in the open ocean that have an appreciable influence on the navigation of ships.

However, when suitable measurements of currents were available, it was found that the results of the theory of Zöppritz were of an entirely different order of magnitude from the observed values; and it appeared that the deflecting force due to the earth's rotation could not be neglected as compared to the other forces, even in a wind-driven current. Thus the elaborate theory, developed by Zöppritz and widely accepted and used by other investigators, though logically sound, proved useless in numerical applications because of errors in his fundamental assumptions; yet it was of value in stimulating further researches and in removing objections previously raised against the wind theory.

According to Hoffman (7, 1884), the variability of ocean currents suggests a correspondingly variable cause, such as the winds. While admitting the importance of the deflecting force due to the earth's rotation in the case of "free" currents, he concluded with Zöppritz that it must be negligible as compared to the other forces in the case of wind-driven currents. Comparing the given surface currents with the given winds, he found on the whole a good qualitative agreement with the friction theory developed by Zöppritz. However, from the assumption used by Zöppritz relative to wind-driven currents, the movement would have the same direction as the winds. But Hoffman found a deflection to the right in certain regions, for example the Guinea Current. He attributed this deflection to the variability of the winds; and he concluded that the current would be driven in the direction of the winds when they were strong, but would be deflected in the same way as a free current when the winds were weak.

In contrast to these examples consider the pioneer work of the Norwegian meteorologist, Mohn (11), who in 1885 synthesized the currents of the Norwegian Sea, including friction, specific gravity, pressure, and winds in his computation. He assumed that the current driven by a wind would cause an inclination of the surface sufficient to balance the deflecting force directed to the right of the motion, and regarded the friction between layers of water as of minor importance. He defined the "wind surface" to be that surface which the sea would assume relative to the general level as a result of the wind-driven currents. Similarly he defined a "density surface," the height of which was computed from serial observations on temperature and salinity. Finally, by combining these two surfaces he obtained the "current-surface," the current being directed along the contour lines with a velocity inversely proportional to the distance between them.

His elaborate investigations involved long and tedious calculations characteristic of most pioneer work. He thus initiated a new era in theories of oceanic circulation and methods of computing the velocity of ocean currents.

Consider now the modern attitude toward the subject of ocean currents. It is generally agreed that they are caused by various external forces, frictional drag of surface winds, friction of a neighboring current, and differences in pressure resulting from differences in specific gravity, caused by evaporation, radiation, and precipitation. The currents are modified by secondary agencies—the deflecting force due to the earth's rotation, and the internal friction of the water.

Friction, the most complex of these factors, has received relatively little attention. However, encouraging results have come from investigations restricted to certain limited regions, the purpose being to work out the corresponding current systems, and the part played by the different agencies. The results of such studies as have been made clearly show that the relative importance of the different agencies varies greatly from one region to another. As to the circulation of the ocean as a whole, the modern view is that there is a slow but very extensive flow of deep water toward the Equator due to differences in specific gravity, while the winds are largely responsible for some of the surface currents but may under favorable conditions give rise to deep currents along the coasts. In the North Pacific there is evidence of a flow beyond the Equator from the Antarctic. In some cases, differences in specific gravity alone, combined with the effect of the rotation of the earth, have been found to account for the whole circulation.

The first task of determining physical and chemical magnitudes becomes more feasible, and can be more accurately and completely executed, with the aid of hypotheses concerning causal relationships between the phenomena, than is possible if only direct determinations are attempted. Thus the objective and theoretical aspects of oceanographic investigations are mutually dependent; available observations render sound theoretical developments possible, which in turn serve directly to increase our knowledge of processes even though they cannot be directly observed.

Collaboration with his father led Professor V. Bjerknes (2) to devote his time mainly to theoretical hydrodynamics. And during about twenty years of concentrated effort in this highly abstract and mathematical field he was not concerned with its application to aërodynamics, meteorology, or oceanography. Accordingly his contribution is of a basic and general type. He thus founded the science of what he calls "physical hydrodynamics," in which the fluids are baroclinic, that is, the density may depend not only upon the pressure but upon any other factors, such as humidity, temperature, and salinity. Later, he considered applications of physical hydrodynamics to practical problems in meteorology and oceanography because in these cases the classical theory of barotropic fluids fails. Circulation in the sea is caused in part by winds and in part by differences in density due to variations of solar radiation, evaporation, and diffusion. In the atmos-

where the density depends upon pressure, temperature, and humidity. While computations of ocean currents due to differences in specific gravity are based upon fundamental principles of mechanics, in particular the Archimedian laws of hydrostatics, practical applications are greatly facilitated by transforming these principles into new and more developed forms especially devised for the purpose.

The fundamental work of V. Bjerknes (1) in "physical hydrodynamics," a theory of fluid motion in which density or its reciprocal, specific volume, may depend not only upon pressure but upon any number of other variables, temperature, humidity, salinity, etc., included such a development, upon which has been based one of the most successful and progressive attempts to apply mathematics to problems of meteorology and oceanography. His students and colleagues, Sandström (1), Ekman (6), Helland-Hansen (15), Nansen, and others interested in physical oceanography, have evolved from his basic contribution practicable methods for computing the direction and velocity of ocean currents from temperatures and salinities observed at a suitable number of known depths and stations. What is known as the Bjerknes circulation theorem, which is expressed by a single differential equation, takes into account the actual distribution of density, the deflecting force due to the earth's rotation and friction.

The simplest and most commonly used special case of the Bjerknes circulation theorem (see Smith, 16) assumes a fully developed current caused by the distribution of pressure corresponding to the observed distribution of temperature and salinity. The deflecting force due to the earth's rotation is proportional to the velocity of the water, is directed to the right of this velocity, and balances the pressure gradient. His circulation theorem expressing this relation then reduces to the following equation:

$$C_n - C_b = \left(\frac{.0195}{2w [\sin \varphi] 10^5} \right) \frac{\Delta_{nb}^{100}}{L} \text{ knots per hour}$$

where $(C_n - C_b)$ is the velocity relative to the bottom, w equals the angular velocity of the earth, L is the horizontal distance in kilometers between stations, φ equals the latitude, Δ_{nb} is the difference in the height of the water above the lowest level, common to two adjacent stations. This difference in height is due to differences in specific gravity at the two stations.

The coefficient $\frac{.0195}{2w [\sin \varphi] 10^5}$ varies only with the latitude and can be read off directly from a table of corresponding values.

From very careful laboratory experiments by Knudsen, Ekman, and others on the physical properties of sea water accurate tables have been prepared indicating the relation of density and its reciprocal specific volume (V) to temperature (T), salinity (S), and pressure (p). Combining these

results with the fundamental equation of hydrostatics that the pressure due to a column of water is proportional to the depth (h), its average density, and the acceleration of gravity (g), a convenient plan has been devised for computing the specific volume *in situ* and the "dynamic depths" of isobaric surfaces ($D = pV$) from temperature and salinity observations. The difference between values of (D) at two stations, and the distance between the stations, determines the pressure gradient. Computations by Wüst (19) of velocities in the Gulf Stream off the Florida coast agreed very well with current-meter observations made by Pillsbury (13), thus demonstrating the practical value of the Bjerknes method.

Another important application of hydrodynamics to physical oceanography is the well-known Ekman (4, 5) theory of wind-driven currents. To Ekman, a student of Bjerknes, belongs the credit of first including in a rigorous mathematical treatment all of the factors essential to wind-driven currents. These factors are the frictional drag of the wind upon the water surface, the deflecting force due to the earth's rotation, and the internal friction due to relative motion within the water.

Under certain conditions that are often realized in practice when applying the Bjerknes theory, the internal friction is of little importance and may be neglected. But in wind-driven ocean currents it is always fundamental, and is caused by irregular eddies involving an interchange of small masses of water. The name turbulence is applied to a motion thus characterized by the presence of irregular eddies. Turbulence may be measured by the mass of fluid exchanged per unit time per unit area between two neighboring units of volume. This quantity has been called "*die Austauschgrösse*" by W. Schmidt, and the "eddy-viscosity" by L. F. Richardson (14) and G. I. Taylor (17) in their investigations of the dynamics of the atmosphere. One of the main difficulties in problems involving eddy-viscosity is its great variability. Little is known about the variation of eddy-viscosity in the sea, but its variation in the atmosphere has been very extensively investigated.

More than twenty-five years ago the observations of Dr. Fridtjof Nansen (12) on winds and surface drift in the North Polar region revealed a definite tendency of the current to deviate to the right of the wind. At his suggestion, Dr. V. W. Ekman, a young mathematical physicist, formulated mathematically the problem of wind-driven ocean currents, using the general equations of the motion of a viscous fluid, in which the deflecting force due to the earth's rotation was included. Also he replaced the coefficient of viscosity by a coefficient of "turbulent friction," the value of this coefficient being determined by applying the solution of his formal equations to field data. Later papers indicate his continued interest in this problem and his elaborate analysis including the effects of latitude, depth of water, neighboring coasts, and topography of the bottom, have furnished oceanographers with the first mathematical equations, applicable to this problem,

that rest upon an adequate physical basis. Certain of his conclusions are briefly summarized here:

1. The surface current caused by wind tends to be deflected to the right of the wind through an angle of 45° in case the ocean is very deep and in the absence of coastal barriers.

2. The velocity of the resulting current varies directly as the wind velocity and inversely as the square root of the sine of the latitude. In general the current velocity is roughly two per cent of the wind velocity.

3. The current at any level below the surface tends to deviate to the right, the lower the level the greater the deviation, and its speed decreases according to an exponential function of the depth. It averages only three or four per cent of its surface value when the direction is 180° from that at the surface.

4. The resultant momentum of this layer of water is directed at right angles to the wind.

5. A difference in level at the surface resulting in a pressure gradient will cause a current to flow at right angles to the gradient, except near the bottom, where the direction turns more in line with the gradient.

6. The thickness of this bottom layer within which appreciable turning of the velocity in the direction of the gradient occurs is about the same as the thickness of the upper layer within which the velocity turns through an angle of 180° . This thickness, called the "depth of frictional influence," varies directly with the wind and inversely as the square root of the sine of the latitude, and averages roughly 100 meters.

7. As the depth of the sea decreases from this "depth of frictional resistance" the frictional effect becomes of relatively greater importance than the effect of the deflecting force. The current tends to deviate less from the direction of the pressure gradients or frictional drag of the wind at the surface.

Some of these principles are illustrated by observations in the North Pacific region. They show that (McEwen, 8), except for one or two months in winter, the ocean winds along the Pacific Coast of North America, from about latitude 20° to 45° , blow toward the southeast or southwest. This agrees with the general relation between winds and isobars. Accordingly, there is a component of the wind parallel to the coast and directed to the south. Therefore, according to Ekman's theory, surface water is carried along the coast and to the right (to the west) of the coast by this component. This gives rise to a lowering of the water level near the coast and an elevation to the west. Therefore, along the bottom, the pressure of the water decreases toward the coast. Accordingly, near the bottom the water flows toward the coast and to the south, thus replacing the water that is being removed from the layer near the surface. In between the surface and bottom layers the flow is directed to the south and parallel to the coast.

Another indirect method of calculating the velocity of ocean currents can be based upon the effect on circulation of the distribution of temperature, McEwen (9, 10). The quantity of radiant energy that passes through a unit area of the sea and is absorbed by a thin layer of water varies directly as its thickness and is assumed to be proportional to the amount of radiant energy that reaches a horizontal surface. Energy leaves this layer because of back radiation and evaporation at a rate approximately proportional to the temperature departure from a constant corresponding to the latitude. The time rate of change of the energy in this volume equals the difference between the rate at which it enters and leaves. If the water flows horizontally, and if there is a temperature gradient in line with the direction of flow, the product of this gradient by the velocity is proportional to the rate at which energy enters or leaves the volume due to the current.

The average of surface temperatures corresponding to a given circle of latitude tends to approximate the "normal" for that latitude as the number of observations increases, if they are so distributed as to be representative. The "normal" temperature is defined as the temperature that would result if there were no current. From estimates of such mean annual temperatures, the seasonal range of temperatures, and solar radiation, an equation has been derived between the temperatures, observed and "normal," at two points along a stream line, its angle with the meridian, and the velocity of the current. The fundamental differential equation is

$$\frac{\partial \vartheta}{\partial t} = BC^{-by} [(a_1 + a_2 x) \cos at + a_3 x + 1] - k(\vartheta - \vartheta_1) - H \frac{\partial \vartheta}{\partial z}.$$

where $k(\vartheta - \vartheta_1)$ is the rate of lowering of temperature due to evaporation and back radiation, ϑ_1 is a temperature for reference not dependent upon the time, ϑ equals the sea-surface temperature, t equals the time, B is the coefficient depending upon the rate at which solar radiation penetrates the surface and its rate of absorption by the sea water, y equals the distance below the water surface, x equals distance north or south of the latitude chosen for reference, H equals the velocity of the current, and the other letters denote physical constants depending upon the rate of radiation. The resulting relation between the observed and normal temperatures for only one point, the observed and normal gradients at that point, and the velocity, has also been derived, and expressed in the form of an equation. The first equation is

$$e^{-R} = \frac{\vartheta - \vartheta_n - G (\cos \psi) (z - z_o) U}{\vartheta_o - \vartheta_{no}}$$

where

$$R = \frac{k}{H} (z - z_o), \text{ and } U = \frac{1 - e^{-R}}{R},$$

ψ is the angle of the stream line with the meridian, G is the normal horizontal temperature gradient along the meridian, k is a physical constant depending upon the rate of cooling, H is the horizontal velocity, $(z - z_o)$ is the distance measured down stream from the point where the observed temperature is ϑ_o and the normal is ϑ_{no} to the point where the observed temperature is ϑ and the normal is ϑ_n . The units are the month, the length of a degree at the equator, and temperatures Centigrade. The value of k is approximately 0.20, and G equals about 0.5. Denoting by G_o the observed gradient along the stream line, the second equation is

$$H = \frac{k(\vartheta_n - \vartheta_o)}{G_o}.$$

The first equation is preferable, but the second may be used in case the data required in the first are not available.

These equations have been applied to numerous temperature observations made in the North Eastern Pacific and the estimates of horizontal current velocities agreed well, on the average, with values obtained by other methods. The same principle has been successfully used to estimate the very much smaller velocity of vertical currents.

In conclusion it is evident that several indirect methods are available for obtaining a knowledge of oceanic circulation from direct observations of temperature, salinity, and wind velocity. Observations of this kind have been carried on extensively for many years, and a very satisfactory field technique has been developed. Although this paper deals exclusively with indirect methods of determining oceanic circulation, important advances have been made in instrumental technique of making direct measurements. An adequate treatment of phenomena so complex and on such a vast scale must involve the co-ordinated use of all available methods.

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IV. THE DISTRIBUTION OF OXYGEN IN THE PACIFIC

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INTRODUCTION

The chemical, biological, and certain geological characteristics of the sea are, in a very large measure, determined and maintained by the oxygen dissolved in the water. In all parts of the ocean and at all depths oxygen is required for respiration by organisms and for the oxidation of chemical substances, derived chiefly from plants and animals. Without oxygen, the products of decomposition would be very different from those formed in the presence of oxygen, and the chemical nature, not only of the water but of the sea bottom as well, would be materially altered. In short, a respiratory system is essential for the ocean as it is for an animal.

Nearly all of what we know in regard to the distribution and circulation of oxygen in the open ocean has been learned from the Atlantic, where oxygen has been studied by a number of oceanographic expeditions. Concerning the distribution of oxygen in the Pacific, on the other hand, very little information is available. The "Challenger" expedition (Dittmar, 5) made about twenty-five determinations of oxygen on subsurface water from various parts of the Pacific, but, because the samples were taken at varying depths and only one at each station, the data are inadequate for any conclusions in regard to the distribution of oxygen. On board the "Planet," Brennecke (2) determined oxygen at several stations between New Guinea and Hongkong, but the information he obtained is not complete even for this limited area because practically no samples were taken below 1,000 meters and even above this depth the number was insufficient. Schmidt (10) reports the oxygen content of the water down to a depth of 1,000 meters at a station occupied by the "Dana" in the Gulf of Panama.

To account for the distribution of oxygen in a body of water it is necessary to consider a number of physical, chemical, and biological conditions. The sea derives the greater part of its supply of oxygen from the atmosphere by absorption at the surface, and variations in the amount absorbed are caused almost entirely by temperature differences, the differences in salinity occurring in the open ocean not being great enough to affect the solubility of oxygen to any appreciable extent. Oxygen is also derived from photosynthetic processes taking place near the surface of the sea. In any case, oxygen enters the sea at or near the surface and is carried to greater depths principally by the water itself, the diffusion of oxygen being too slow to be of any practical significance.

It is obvious, then, that one of the most important factors in determin-

ing the distribution of oxygen in the sea, except in a narrow surface layer, is the circulation. Our knowledge of the circulation of the water in the Pacific is very incomplete, but from certain investigations that have been made and by assuming that the conditions in the Pacific are somewhat similar to those prevailing in the Atlantic, for which more complete information is available, certain generalizations may be made. For our present purpose the sea may be regarded as consisting of two layers of water: Near the surface a relatively thin layer in which turbulence caused by wind and temperature changes occurs, and below this and extending to the bottom of the ocean a more extensive layer which is practically isolated from the overlying atmosphere. For these two layers Defant (4) has used the designations "trophosphere" and "stratosphere," respectively.

In the upper part of the trophosphere the water is mixed more or less thoroughly by the mechanical action of the wind, resulting in uniform temperature conditions down to a depth which varies with latitude, season, meteorological conditions, and other factors. In this layer the oxygen also may be expected to be uniformly distributed, although irregularities may occur because of variations in the biological conditions.

Immediately below this zone of uniform temperature is found the thermocline or discontinuity layer, which is characterized by a rapid decrease in temperature with increasing depth. The thickness of this layer varies with a number of factors, but in the open ocean it usually extends over several hundred meters. Mixing of water from different levels is caused, according to McEwen (9), chiefly by convective currents which result from cooling of water by evaporation at the surface and the sinking of the heavier particles of cool water. McEwen also found that near the California coast this type of circulation is effective to a depth of about 100 meters. Along this coast, however, conditions are somewhat abnormal because of upwelling of cold subsurface water which would naturally lessen the distance that the cool particles from the surface would descend. It is probable that in areas where upwelling does not occur, convective currents may extend to considerably greater depths, possibly to 500 meters or more. It is obvious that particles of water sinking from the surface will carry with them any oxygen that they contain. Since the intensity of the convective movements will naturally decrease as the temperature becomes lower, i.e. as depth increases, the amount of oxygen transferred will similarly decrease. It is probable that other forms of turbulence transport some oxygen to even greater depths than do the convection currents, but it is certain that at great depths the amount of oxygen received from the atmosphere or the upper water strata is so small as to be negligible.

The stratosphere is characterized by nearly uniform physical and chemical conditions. However, here, as well as at higher levels, horizontal currents exist, and it is by such currents that oxygen is carried to all depths

of the sea. Where the depth of water is sufficiently great there are usually two or more of these currents flowing in the same or different directions. They originate probably in the polar or subpolar regions, where, because of the low temperature, the water contains a relatively large amount of oxygen. The abyssal water would consequently be expected to be well supplied with oxygen, and in the Atlantic this has been found to be the case (cf. Wattenberg, 11).

Oxygen in the sea is consumed at all depths by organisms and in the oxidation of organic material. It cannot be definitely stated at what depths consumption is the greatest, but it is certain that the animals are relatively more abundant at the higher levels where their supply of food, namely phytoplankton, is found. Here, also, some of the conditions necessary for oxidation are most favorable. Oxidizable organic material, which is derived chiefly from the organisms, is probably most abundant, and the higher temperatures make possible a higher rate of oxidation than at lower levels. However, in this connection it should be stated that Harvey (6) found, in water samples obtained off Plymouth at a station where the depth of water is 70 meters, that hydrogen peroxide is reduced more rapidly by bottom water than by surface water, although the latter contains much larger quantities of organic material. Harvey attributes the slower rate of oxidation in the surface water to the presence of organic substances which inhibit the action of a catalyst, probably an organic compound of iron, which accelerates oxidation in sea water. From this it appears probable that the decomposition of dead organisms takes place largely at some distance below the point at which they originate.

The present report is based on oxygen determinations made at the mouth of Monterey Bay by Bigelow and Leslie (1), off the coast of Southern California by Leslie and Moberg (7), and between San Francisco and the Hawaiian Islands on board the "Carnegie" by the author. In making all these determinations the well-known Winkler method was used. The details of the procedure were the same in all cases, and the potassium dichromate solutions used for standardizing the sodium thiosulphate were checked against one used at the Scripps Institution, thus making the results strictly comparable. In connection with the oxygen studies, the temperature and salinity and other chemical constituents were also determined, but only the temperature is considered in this article because the solubility of oxygen is not greatly affected by chemical conditions. Adequate information in regard to the biological conditions is not available for any of the oxygen series.

RESULTS AND DISCUSSION

Of the oxygen data here considered, only those obtained on board the "Carnegie" can be regarded as representing typical off-shore conditions.

Between the California coast and the Hawaiian Islands the "Carnegie" occupied ten stations, and at each station about twenty samples were collected from various depths for oxygen determinations. The results for two of these stations are shown in Figure 1. One station, No. 137, was located at latitude $24^{\circ} 01'$ and longitude $145^{\circ} 33'$, or about 1,500 miles approximately southwest of San Francisco, and is typical of the region traversed west of longitude 133° , both the temperature and oxygen curves closely resembling those for six other stations not shown in the figure.

For Station 137 the figure shows a layer of water of high and uniform temperature extending from the surface to a depth of 25 meters. The thermocline, or zone of rapidly decreasing temperature, occurred between 25 and 500 meters. Although the temperature change between 500 and 700 meters was slight, these depths should, perhaps, also be included in the thermocline, or at least in the trophosphere, because the decreasing rate of temperature change indicates the existence of vertical circulation. At 700 meters the temperature was $5^{\circ}17'$ and at subsequent depths decreased at a slow but constant rate.

In regard to the oxygen content, the main facts to be noted are: (1) nearly complete saturation at the surface; (2) higher, both absolute and relative, quantities from 25 to 200 meters; (3) a slow decrease between 200 and 400 meters; (4) a rapid decrease between 400 and 700 meters; (5) a minimum at 700 meters; and (6) a slow and uniform increase below 700 meters. At the surface the oxygen content was 4.63 ml. per liter, or 96 per cent of total saturation. At the other nine stations the degree of saturation ranged from 96 per cent to 100 per cent, but the fluctuation in the absolute values was comparatively greater, or from 4.63 ml. to 5.69 ml., and correlated with the temperature. The increase in the oxygen content below the surface to a maximum of 5.04 ml. at 75 meters was not due to the lower temperature, as shown by a similar increase in the relative concentration which reached the high value of 101 per cent at 50 meters. The increase in the oxygen content was no doubt due to the liberation of oxygen by photosynthetic organisms.

The high oxygen values occurring between the photosynthetic zone and 400 meters may have been due either to turbulence or to a low oxygen consumption, but the temperature gradient indicates that turbulence may have been the chief cause.¹ The more rapid decline in oxygen values between 400 and 700 meters was associated with a more constant temperature which indicates that at these depths mixing was less intense.

The minimum quantity of oxygen was found at all stations at 700 or 800 meters. At the station under consideration the smallest quantity of oxygen

¹ Data obtained since this was written indicate that a subsurface current was probably responsible for the change in the oxygen gradient at 400 meters.

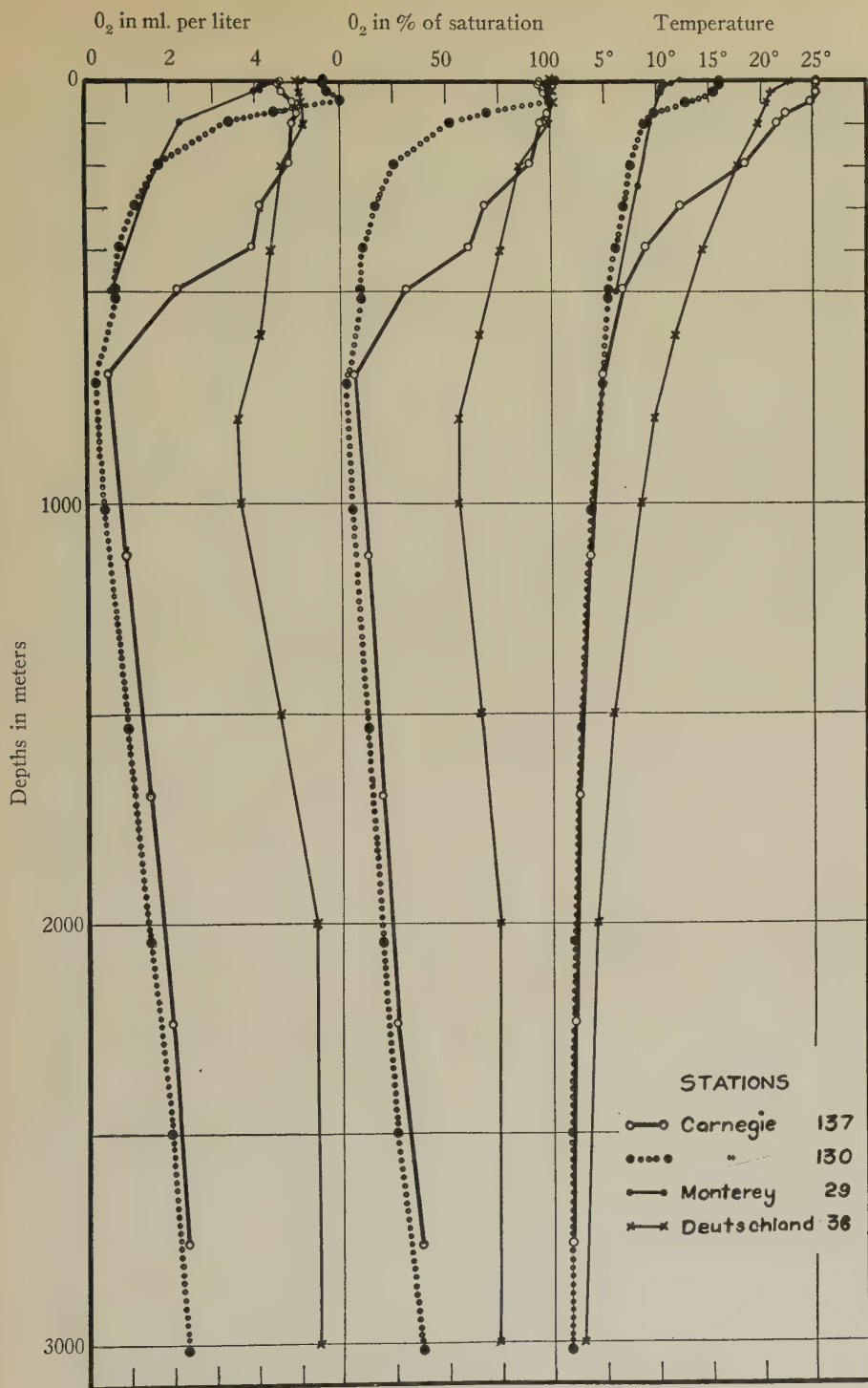


FIG. 1.—Vertical distribution of oxygen and temperature

was 0.53 ml., or 7.6 per cent of total saturation. For all the stations the minimum values ranged from 0.21 ml. to 0.96 ml., or from 3 per cent to 12 per cent. Several causes probably combined to produce this zone of low oxygen content. The depth at which it occurred apparently marks the boundary between the trophosphere and the stratosphere, i.e., the lowest level to which appreciable quantities of oxygen may be transported from the atmosphere. This assumption is corroborated by the temperature curve, which shows a change in the temperature gradient at 700 meters. Oxidation undoubtedly also played a part in reducing the oxygen content. It would appear that the consumption of oxygen is greatest from 400 meters to some distance below 700 meters and this possibility is not contradicted by the results obtained by Harvey (see above, p. 71).

An outstanding feature of the water below 700 meters was the constancy of the rate of change in the temperature and the oxygen content. At 2,760 meters, the lowest depth represented in the figure, the temperature had decreased to 1:77 and the oxygen had increased to 2.36 ml., or 31 per cent. Other observations taken at 500-meter intervals down to 4,770 meters show a slight decrease in the rate of temperature and oxygen change. At 4,770 meters the temperature was 1:53 and the oxygen content 3.45 ml., or 45 per cent. This is the highest oxygen value obtained below the minimum zone at any station.

As has been previously stated, the water below 700 meters probably receives no oxygen from the atmosphere above and the oxygen contained below that level was absorbed in some other locality, where this water descended from the surface. The descent probably took place in a region where the surface temperature is considerably lower than it is at Station 137, and there has thus been a marked loss of oxygen, particularly near the minimum layer where practically nothing remains. This great loss indicates either a high rate of oxidation or a long period of separation from the atmosphere or both.

The other station, No. 130, was located at Latitude 37° 05' and Longitude 123° 43', or about sixty miles southwest of San Francisco, and represents a locality materially affected by upwelling subsurface water. At this station, the temperature at 700 meters and below was almost identical with that at corresponding depths at Station 137, whereas the oxygen content averaged about 0.5 ml. lower. At depths above 700 meters, however, there was very little similarity between the two stations, the main point of difference being that at Station 130 the rapid decline in temperature and oxygen values began much nearer the surface than at Station 137. At Station 130, the temperature at the surface was 16:24, whereas, according to McEwen (8), the normal temperature for this latitude is about 20°. It has long been known that the low temperatures prevailing along the California coast are due to upwelling of cold subsurface water which subse-

quently flows away from the coast in a southwesterly direction. Both the temperature and the oxygen gradients give evidence of an upward movement of deep water of low temperature and oxygen content. The most rapid decline in temperature occurred between 25 and 75 meters, and that in oxygen between 50 and about 200 meters. Between the surface and 50 meters the saturation of the water with respect to oxygen was almost exactly 100 per cent (99.7 per cent to 100.5 per cent) and the absolute quantities were 5.69 ml. at the surface and 6.05 ml. at 50 meters, the latter value being the maximum for this station. The lowest quantity of oxygen found was 0.24 ml., or 3.4 per cent of total saturation, and occurred, as in the case of Station 137, at 700 meters.

Evidence of an even greater amount of upwelling is shown by the data reported by Bigelow and Leslie (1) for a station (Monterey 29 in Figure 1) located in a submarine valley at the mouth of Monterey Bay. The depth of water at this station was about 700 meters, but no samples were taken below 500 meters. Although somewhat farther south than "Carnegie" Station 130, the temperature at the surface was only 12°39', or nearly 4° lower than at Station 130. A rapid decline in temperature began immediately below the surface but continued to fifteen meters only and between 100 and 500 meters the temperatures were nearly the same as at corresponding depths at Station 130. The quantity of oxygen at the surface was only 5.20 ml., or considerably below saturation, and no higher values were found at lower levels. A rapid decline in oxygen began immediately at the surface and continued to 100 meters. At 250 and 500 meters the oxygen content was the same as at Station 130, the quantity obtained at 500 meters being 0.63 ml.

Oxygen was determined by Leslie and Moberg (7) at a number of stations located at from 10 to 50 miles from the Southern California coast. The data for these stations are not presented here, but it may be stated that below 200 or 300 meters the conditions resembled those at Monterey Bay and at "Carnegie" Station 130. Above these depths, the oxygen values, as well as the temperatures, were somewhat higher and the gradients less steep than at the two stations just mentioned, indicating a lesser amount of upwelling. In the photosynthetic zone the water was nearly always supersaturated with oxygen.

All the stations along the coast ("Carnegie" 130, Monterey 29, and the Southern California stations) thus show a much lower oxygen content in the upper 500 meters or more than do the stations (such as "Carnegie" 137) representing more typical oceanic conditions. This is probably due both to a greater abundance of organisms and other organic material along the coast and to an upward movement of deeper water. The fact that the depth at which the oxygen content was at a minimum did not vary with distance from the coast indicates that the depth at which the greatest

amount of oxidation takes place is practically constant for the area investigated.

In order to facilitate a comparison of the distribution of oxygen in the Pacific with that in the Atlantic, the temperature and oxygen curves for a station occupied by the "Deutschland" (Brenneke, 3) are shown in Figure 1. This station was located at Latitude $27^{\circ} 46' N.$ and Longitude $27^{\circ} 36' W.$, i.e., at a latitude between the latitudes of the two "Carnegie" stations.

The oxygen curve for the Atlantic station shows very little resemblance to the other oxygen curves. In the upper 400 meters the Atlantic station contained about the same quantity of oxygen as did Carnegie Station 137 but at lower levels considerably more. The minimum oxygen value in the Atlantic was 3.5 ml. at 800 meters as compared to 0.24 ml. and 0.53 ml. at the two Pacific stations. Near 3,000 meters the figures were: Atlantic 5.5 ml., Pacific 2.4 and 2.4 ml. Differences of the same order of magnitude are shown for all depths below 500 or 600 meters. It may be noticed that at the Atlantic station the oxygen content at 2,000 and 3,000 meters was higher than at the surface, a condition not so far reported for the Pacific.

Mention has been made of oxygen determinations made by Schmidt (10) at a station in the Gulf of Panama. He reports that at this station the water between 400 and 500 meters "contains practically no oxygen at all." At a station occupied a few days previously on the Atlantic side of Panama the oxygen content in the same stratum was between 40 per cent and 50 per cent of complete saturation. A low oxygen content below a few hundred meters in the Pacific was indicated also by the investigations made by the "Challenger" and the "Planet" referred to above.

On the other hand, the oxygen curve for the "Deutschland" station shown in Figure 1 appears to be representative for other parts of the Atlantic, at least for depths below the zone of minimum oxygen content. According to a recent summary by Wattenberg (11) of the distribution of oxygen in the Atlantic, the oxygen content at all depths below 1,800 meters in all parts of this ocean contains 5 ml. or more of oxygen per liter. This applies even to the tropical Atlantic, where the oxygen content at about 500 meters is often less than 1 ml. per liter.

SUMMARY

Recent studies have been made of the distribution of oxygen in the Eastern Pacific, between Latitudes $20^{\circ} N.$ and $40^{\circ} N.$ and from the coast to about 2,000 miles to sea. These studies show that in this area the oxygen content decreases from nearly complete saturation at the surface to a minimum of less than 1 ml. per liter at a depth of about 700 meters

and then increases slowly toward the bottom, the highest amount of oxygen obtained in the deep water being 3.45 ml. per liter. Near the coast, the oxygen gradient in the upper few hundred meters of water is considerably modified by the upwelling of subsurface water. Comparing the oxygen content of the Pacific with that of the Atlantic, it is found that in the latter ocean the minimum oxygen values in most latitudes and the oxygen values at greater depths in all areas are much higher than in the Pacific. This difference cannot be satisfactorily explained until further information concerning the circulation and the biological conditions in the Pacific is available. One possible explanation is that in the Pacific the consumption of oxygen is greater than in the Atlantic, but a more probable one is that the deep water in the Pacific may be removed from contact with the atmosphere for longer periods than that in the Atlantic.

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V. A PROGRESS REPORT ON IONIC RATIOS AND SPECIFIC GRAVITY OF SEA WATER

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A review of the literature for the past seventy years reveals a large number of analyses of sea water from all regions of the world. To make the data comparable, corrections for different gravimetric factors used by the several investigators must be made, owing to variations in atomic weights from time to time during this period. The accuracy of some of the analytical results may be questioned because of obviously poor technique or the use of doubtful methods of analysis. However, the data in general indicate that sea water is of very nearly uniform composition and varies only as to the state of dilution.

Postulating the constancy of composition of sea water as a working hypothesis, Forch, Sorenson, and Knudsen (3) thirty years ago began their classical investigations, which eventually became part of the foundation of modern oceanographical studies. The constant relationship between chlorinity, density, and an arbitrary unit for total solids, commonly known as salinity, was demonstrated by them.

While the constancy of composition of some of the major components of sea water has been demonstrated within certain limits, it must be borne in mind that the sea is a system of tremendous complexity. Vast numbers of actions and reactions of a physical, chemical, and biological nature are continually taking place. Substances in suspension and in solution are constantly being poured into the sea. All the myriad forms of plant and animal life bring about a complicated series of changes. The formation and melting of sea ice, atmospheric effects, volcanic activity, and slow geologic processes, all exert an influence upon the composition of the waters of the seas. In spite of the vastness and complexity of innumerable factors, a point of balance or equilibrium persists. The resultant of any effect when compared with the entire mass of water may be very small, hardly detectable for the predominating ions, but readily measurable in many instances for those occurring only in very low concentrations. Thus an increase or decrease of 0.1 milligram per liter of the sulfate ion in a water mass containing 2,500 milligrams per liter of the same ion could hardly be measured, but an increase or decrease of the same amount of the phosphate or ammonium ions in sea water would readily be measured and would undoubtedly be described as a most decided change. In other words, the predominating ions in sea water appear to be present in quantities that are definitely proportional to one another, but those existing in very small concentrations tend to vary materially according to conditions.

In the chemical laboratories of the University of Washington, samples of the waters of the northeast Pacific and the Puget Sound regions have been studied (5, 6) and ratio constants for several of the predominating ions, with chlorinity as unity, have been determined. These investigations were carried out with considerable analytical refinement on a number of samples. It was found that, regardless of the season of the year or the locality or depth at which the samples were taken, the ionic ratios of sulfate, calcium, and magnesium, with chlorinity as unity, were remarkably constant. These statements, however, do not apply to waters that have been considerably diluted by drainage from the land or to stagnant areas devoid of circulation. The ionic ratio constants determined to date are

$$\text{SO}_4/\text{Cl} = 0.1396$$

$$\text{Ca}/\text{Cl} = 0.02150$$

$$\text{Mg}/\text{Cl} = 0.06692$$

The constants for sulfate and calcium agree very well with the general averages calculated from the data of other investigators. The magnesium constant is somewhat lower because advantage was taken in its determination of the refinements described in the recent work of Epperson (2). These insured the complete removal of potassium from the magnesium ammonium phosphate, precautions not observed generally in previous analyses of sea water.

Knowing the chlorinity and the ionic ratios, the composition of any normal sea water may be readily ascertained on either a volume or a weight basis for the sample. A direct analysis of a sea water that has been subjected to abnormal conditions may give ratios that vary from those of normal and thus a measure of the abnormality may be obtained by noting the extent of the deviation. The ratio constants should be of value in the study of selective adsorption of the predominating ions of sea water by various substances. In like manner measures of the degree of pollution of sea water by certain industrial wastes may be made. The mechanisms of dilution by river discharge and the effects of sedimentation resulting therefrom should be measurable and explainable by noting variations in the ionic ratios when the composition of the diluting water is known.

In connection with river discharge attention may be called to the observations of Clark (1), who showed that the order of abundance of the cations occurring in fresh water are: calcium, magnesium, and sodium. The latter is present in the least amount. In sea water, while the concentrations are much greater, the order of abundance is reversed, calcium occurring in the smallest quantities. The anions, in order of abundance in river waters, are the carbonates, sulfate, and chloride, and again the order is reversed in sea water. A program has been outlined for studying the effects upon the ionic ratios by natural dilution.

As the result of the investigation of the sulfate-chlorinity ratio of the waters of the northeast Pacific, Professor Martin Knudsen of the Hydrographical Laboratories of Copenhagen has requested that the study be extended so as to include all other seas of the world. Through the courtesy of Professor Knudsen, the International Council for the Exploration of the Sea, the International Fisheries Commission, the Carnegie Institution of Washington, and the Scripps Institution of Oceanography, sea waters from various regions of the world, taken from different depths, are now being analyzed in the author's laboratories in collaboration with Henry E. Wirth, Calvert C. Wright, and William Johnston. Professor Knudsen has also requested that accurate density determinations of these waters be made particularly at 0° as questions have arisen from time to time as to the applicability of the Hydrographical Tables for waters of the Mediterranean Sea and the Red Sea.

For the density determinations two pycnometers made of transparent quartz and having a capacity of 100 milliliters have been secured. The substitution of quartz for glass reduces considerably the errors arising from thermal expansion or contraction of the apparatus. The pycnometers are similar in many respects to the one originally used by Professor Knudsen but with the refinements recently recommended by Parker and Parker (4). The latter investigators also made a study of the methods for maintaining a constant temperature of zero degrees, and that recommended by them, i.e., the use of well-drained ice shavings, is being utilized. It has been found that checks of one part in a million are easily obtainable. The results of the study we hope to report to you at a future meeting.

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VI. A SUMMARY OF STUDIES OF THE PHOSPHATE CONTENT OF THE WATERS OF THE SAN JUAN ARCHIPELAGO

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The Puget Sound region offers an excellent opportunity for the study of the physical and chemical characteristics of sea water under remarkably different conditions. There are many estuaries with rather varied topographies in the Sound proper, while among the San Juan Islands are many lagoons of interesting formation. A number of small rivers flow into the Sound and produce local areas that show wide seasonal variation and offer an excellent opportunity to study changes produced in sea water as the result of natural dilution. A very interesting effect of river discharge, as shown by Lucas and Hutchinson (5, 6, 9, 10), is that of the Fraser River of British Columbia. It empties into the Strait of Georgia just north of Puget Sound and is by far the largest river in this region. For a short period during midsummer the surface waters of the San Juan Archipelago are noticeably affected by the Fraser River. Unpublished data of Professor Shelford, of the authors, and of Professor R. C. Miller show that there is no similar effect of the rivers emptying into Puget Sound upon the waters of the Archipelago.

A very decided and marked upwelling of ocean water must either occur off the coast of the state of Washington, analogous in some respects to that first pointed out by McEwen (11) for the waters off the California coast, or else in the Strait of Juan de Fuca as the result of tidal currents striking the land barriers. It is this upwelled water, rich in nutritive material, that comprises that of Puget Sound and the adjacent region. Evidence of this upwelling is shown particularly in the phosphate content of the water but may likewise be shown by the study of other nutritive material. The present paper deals with a summary of results obtained during 1928 and 1929 at the Puget Sound Biological Station, and reviews the results of about 400 analyses of water for the phosphate ions.

The determinations were made using Atkins' (1) modification of Denige's method with the employment of a Duboscq colorimeter. This method gives only the phosphorus in the form of phosphate dissolved in the water and consequently does not include phosphorus of an organic nature or that existing in a lower form of oxidation in inorganic compounds. Any arsenates present in the water are reported as phosphates. In practically all cases the analyses were performed within an hour or two after the samples were obtained. Care was taken in the preparation and checking of new standards from time to time, and all reagents used were tested for phosphates.

Many investigators have shown the general effect of living organisms upon the concentration of these ions. All data published emphasize this important fact, but instances may be found in the literature where the data may exaggerate actual conditions. This is particularly true in waters that show wide differences in chlorinity. Under such circumstances the effects of dilution upon the concentrations of phosphates have not been considered. If the data were reported on the basis of chlorinity or salinity as unity or discussed from this standpoint, different conclusions might be reached. Thus the phosphate-chlorinity ratio may reveal interesting conditions and may tell much concerning the nature and the source of the water.

The one objection that may be raised to the usage of the phosphate-chlorinity ratio is the original phosphate concentration of the diluting water. The phosphate ions generally occur in quantities considerably greater in ocean water than that found in the fresh-water streams of the Pacific Slope. The phosphate content of a fresh-water stream is obviously dependent upon the geologic formation of the region drained by it. Several analyses by one of us show only slight traces occurring in three rivers of the Puget Sound region, and Clark (2) and Kemmerer *et al.* (8) report only very small quantities for the fresh-water lakes of this region and in several cases only minute traces. From these rather meager data it may be concluded that dilutions of ocean water by land drainage may have only a small effect upon the phosphate-chlorinity ratio of this region.

Much of the phosphate data for European waters reported by Harvey (4) for water masses near the surface show concentrations very much smaller than those obtained in the Puget Sound region. Harvey states that the phosphate concentration increases with depth, and some of the data quoted by him show from traces to 0.067 mg. PO_4 near the surface to as high as 0.400 mg. PO_4 at great depths. All of the samples analyzed from the San Juan Archipelago showed an average of 0.189 mg. PO_4 . A minimum of 0.032 mg. PO_4 was obtained in an estuary where the waters were particularly rich in diatoms. The surface waters averaged about 0.188 mg. for an entire year, while in the deeper layers at 200 and 300 meters values approaching 0.240 mg. were obtained. In many cases, however, a very little difference between the surface and the samples at 100 and 150 meters was observed, especially during flood tides. The average phosphate-chlorinity ratio was found to be $\text{PO}_4/\text{Cl} = 0.0110$, where PO_4 is milligrams per liter and Cl is chlorinity per liter.

The high concentration of phosphate found by Lucas and Hutchinson in the waters of the Strait of Georgia was attributed by them to discharge from the Fraser River. As no data had been reported prior to their papers for the Puget Sound region, just south of the locality in which they worked, and as their results were higher than any cited in the

literature, their conclusion appeared logical. The very turbid nature of the Fraser River evidently made phosphate determinations impossible, for only one result on this water was given and this appears to be abnormally high for river waters of this particular region.

The present authors do not agree with the conclusion of Lucas and Hutchinson, but are of the opinion that the high phosphate content of the waters of the Puget Sound and adjacent regions is chiefly the result of upwelling of the ocean water either off the Washington and British Columbia coasts or in the Strait of Juan de Fuca. A detailed report giving all of our data will appear in the near future in the *Publications of the Puget Sound Biological Station*. Further studies of water off the Washington Coast are contemplated.

The evidence for the high phosphate concentration of these regions as the result of upwelling is as follows:

1. The waters of the San Juan Archipelago and the Strait of Juan de Fuca are characterized by a very rich diatom growth and have been classified into three types of water designated by Gran and Thompson (3) as surface, intermediate, and ocean water. In general these waters have a lower temperature and higher chlorinity than those waters immediately to the north studied by Lucas and Hutchinson. The surface and intermediate waters, the latter in particular, show pH readings, silicate and phosphate content, and temperatures analogous to the data obtained by Moberg (12) off the California Coast at depths of 100 and 150 meters, but with lower chlorinities.

2. A number of phosphate-chlorinity ratios for the diluted waters of the Gulf of Georgia calculated from the graphs given by Lucas and Hutchinson are analogous to those obtained in the waters of the San Juan Archipelago. On the other hand there are a number of these ratios that become less as the mouth of the Fraser is approached, whereas they should become greater if the Fraser discharges quantities of phosphate.

3. When the waters of the San Juan Archipelago at the Puget Sound Biological Station were influenced during the week or two of midsummer by the flow of diluted waters from the Gulf of Georgia (7, 13), the phosphate content was low but the phosphate-chlorinity ratio was average. On the other hand, when the amount of river discharge is the lowest in the fall and winter months, the highest chlorinities of the year are obtained, indicating ocean water. Likewise the phosphates are the highest, and so are the phosphate-chlorinity ratios.

4. In February, Hutchinson *et al.* report a phosphate content as high as 0.222 PO_4 at the surface. This is comparable with 0.314 mg. PO_4 obtained at the same time at the Puget Sound Biological Station during a flood tide and when the influence of the Fraser was nil.

5. Analyses of the waters of the Strait of Juan de Fuca just before

the strong incoming tidal current strikes the island barriers and shallower channels show a phosphate-chlorinity ratio much higher than the average and much higher than any reported by Lucas and Hutchinson.

CONCLUSION

The waters of the Puget Sound region are very rich in phosphates, the result of upwelled ocean water. The high phosphate concentration cannot be attributed to river discharge.

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VII. AN OCEANOGRAPHIC SURVEY OF THE STRAIT OF GEORGIA¹

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The following is an outline of the more complete account (with charts) to be published in *The Canadian Research Journal*, Ottawa.

Significant features of the Strait of Georgia.—The Strait of Georgia is approximately 150 miles long and has an average width of twenty miles. It is interrupted by two large islands, Texada and Lasqueti. On the northeast it is bounded by the mainland of the Province of British Columbia and the state of Washington and on the southwest by Vancouver Island. A series of islands separated by narrow passes (Gabriola, Porlier, and Active) run parallel to the shore of Vancouver Island toward its southern extremity to form the narrow channels, Trincomali and Stuart. The Strait of Georgia is connected with the Pacific Ocean by narrow channels, namely Discovery Passage, Okisollo Channel, and Sutil Channel at the northwest, and by Boundary Passage and Rosario Strait at the southeast extremity.

The Fraser River, which drains a large portion of the Province of British Columbia, flows into the strait about twenty miles north of Boundary Passage. A number of deep inlets occur on the mainland side of the strait, such as Howe Sound, and Burrard, Jervis, Bute, and Toba Inlets, into which flow glacial streams. Many rivers flow from Vancouver Island, having their sources in upland lakes.

The tides are characterized by a remarkable inequality in successive ebb and flood. The flood tide from the Strait of Juan de Fuca flows northward almost to the northern limit of the Strait of Georgia, where it meets the flood tide entering from the north through Johnstone Strait and Discovery Passage. At the south, the flood tide flows one hour longer through Rosario Strait than through Haro Strait and the reverse is the case with the ebb tide. A great swirl extending from these passes to Boundary Bay and within a few miles of the southern outlet of the Fraser River results from this tidal phenomenon.

Surface salinity.—The surface salinity within the Strait of Georgia varies greatly and is dependent upon a number of factors, among which may be mentioned:

a) The size of streams entering the strait and the consequent amount of fresh water. At the mouth of the Fraser, at the head of Howe Sound and of Bute Inlet the surface salinity during July and August is less than

¹ Read by W. A. Clemens.

2 g. chloride per liter. At a similar distance from Powell River the salinity is 5 g. Cl per liter and near Comox and Qualicum Rivers the salinity is 12 g. Cl per liter. The latter rivers not only are smaller but are exposed to the sweep of the tides.

b) The salinity varies in inverse proportion to the distance from the mouth of fresh-water streams, but this proportion may be modified by other conditions. At a distance of thirty miles northwest from the mouth of the Fraser the salinity is 12 g., whereas the same salinity is attained at a distance of ten miles directly opposite the Fraser River mouth. This difference may be explained on the basis of tidal currents. The salinity at the head of Howe Sound and of Bute Inlet is less than 2 g. Cl per liter and is approximately 9 g. at the mouth of each of these arms. In the former case the distance is twenty-five miles, whereas in the latter it is forty miles. Within the arm the fresh water is not subject to the same degree of mixing with the water of higher salinity carried by the tides. Throughout the greater part of the strait the summer influence of the streams is such that the surface salinity does not exceed 14 g. Cl per liter.

c) During the summer there is a remarkably stable epilimnion produced primarily by the low salinity of the surface water and increased in stability, as a result of radiant energy upon temperature and specific gravity. This upper layer of low salinity is maintained in spite of currents produced by winds and tide, but varies to some extent according to the degree of mixing brought about by such currents.

d) The flood tides from the ocean contribute water of high salinity. From Boundary Pass to the mouth of the Fraser, a distance of twenty miles, there is a change in salinity of from 16 to 2 g. Cl per liter. At Cape Mudge entrance to Discovery Passage the salinity is 16+, while five miles eastward it is 13 g. Cl per liter.

e) The most marked changes in salinity are observed at the opposite ends of narrow passes. Active Pass, whose length is two miles, shows a change of more than 2 g. per liter and the narrows to Burrard Inlet illustrates a change of from 10 to 14 g. Cl per liter in a distance of two miles. The stable upper layer is conserved in the strait by a sliding back movement as the water is piled up at the entrances to such passages.

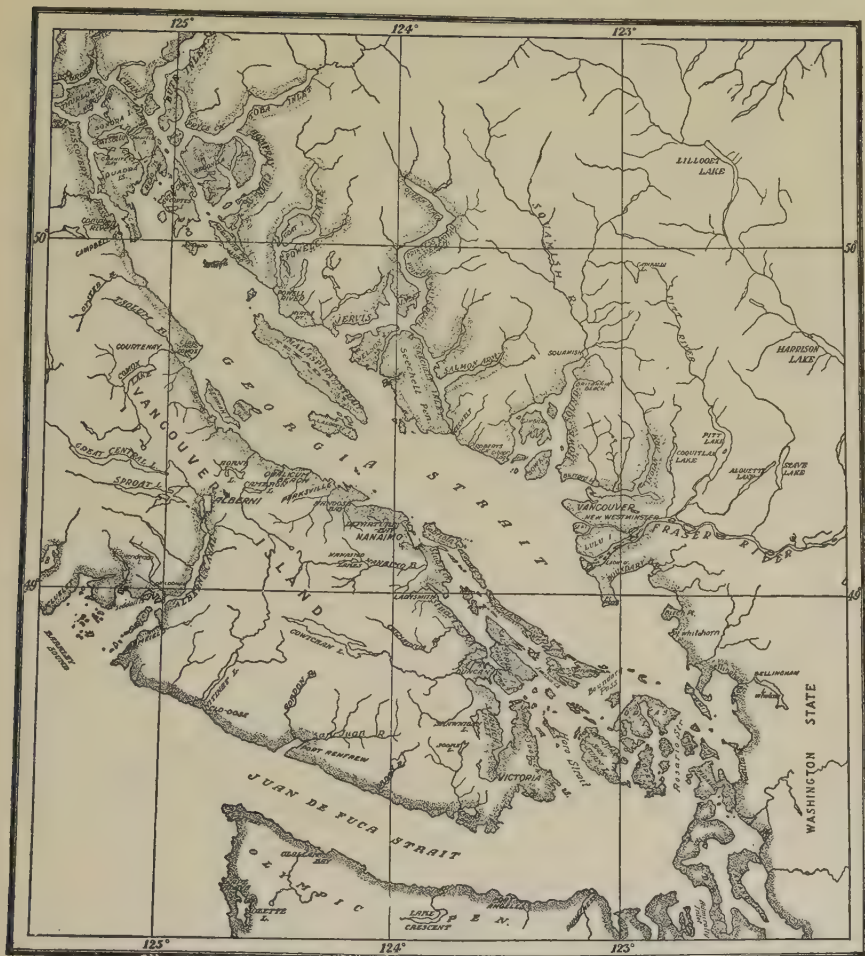
Surface temperatures.—During the summer the greater part of the surface water of the Strait of Georgia shows a remarkably high temperature which frequently exceeds 20°C. The temperature is dependent chiefly upon the following factors:

a) The temperature of the water at the time it enters the strait. During the summer the Fraser River water enters at a temperature from 15° to 16°C., and the rivers from Vancouver Island have a similar temperature. The Squamish River enters at 9° to 10°C. and the rivers at the head of Bute, Toba, and Jervis inlets have a temperature of 5° to 10°C. Sea

water enters from the Strait of Juan de Fuca at 12° to 13°C . and from Johnston Strait at 9° to 10°C .

b) Air temperature, which during the summer tends to increase the water temperature.

c) Evaporation, which tends to lower the temperature, especially when the rate is increased by wind.



Map of the Strait of Georgia

d) Radiation. This is the factor of greatest importance, and its influence is dependent upon the stability of the epithalassa. The areas of highest temperature are some distance from the mouths of rivers. Beginning ten miles from the mouth of the Fraser and extending over a triangular

area twenty miles each way is an area whose temperature approximates 22°C. during the summer months, and even at a distance of one hundred miles northwest of the Fraser the temperature is 2° higher than when the Fraser River enters the strait. Although the glacial water from Bute and Toba inlets has a very low temperature, as it enters, owing to its stability and consequent exposure to radiation the temperature becomes 21° at Savary Island near the north end of the Strait of Georgia.

Distribution of phytoplankton.—The areas of maximal quantity occur where mixing of river and sea water results in a composite. Most notable are the areas north and south of the Fraser River at distances of approximately thirty miles north and twenty miles south, and opposite the Fraser River mouth in Trincomali and Stuart channels. The smaller rivers, such as Powell, Comox, and Qualicum, have smaller areas of high diatom content at relatively short distances from their outlets. Diatoms are not abundant in the inlets such as Toba, Bute, and Jervis, except near the mouth, where mixing of fresh water and sea water takes place. The areas of lowest diatom content at the surface are near the mouths of rivers, such as the Fraser, and at the other salinity extreme, such as at the entrances of Haro and Rosario Straits and of Discovery Passage. It is evident that both the river water and the sea water contribute toward conditions favorable for the growth of phytoplankton. The exact nature of this contribution is discussed in other papers.

VIII. SOME ASPECTS OF THE PHYSICAL CHEMISTRY OF PERMEABILITY TO IONS

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In the last few years artificial membranes have been developed whose permeability presents so many striking analogies with that of living cells that critical consideration of their physical chemistry and its bearing on cellular permeability is most desirable.

The pioneer investigations which led to this recent work were not directed primarily to the study of permeability but rather to establishing the laws governing biological electromotive forces. In the course of this work Loeb and Beutner (13) established the fundamental fact that the intact cutinized epidermis of an apple yields an e.m.f. when it separates two solutions of a given electrolyte of different concentrations, or two solutions of like concentration but differing in the identity of the cation.

At this point the investigation was taken up after some years by Michaelis and his co-workers (15). After verifying in all its essential details the work of Loeb and Beutner they succeeded in finding a reproducible artificial membrane which showed in still simpler form the behavior characteristic of the apple epidermis. This membrane was made by allowing essentially complete drying, i.e., evaporation of the ether and alcohol, of collodion membranes before bringing them into contact with water. Membranes so prepared allowed no salt to pass through them from a solution of a strong electrolyte into distilled water; they would allow sodium and potassium, for example, to pass through them by exchange between solutions initially containing sodium and potassium salts, respectively; but anions were under all conditions prevented from passing through in perceptible amounts. When the more perfect of such membranes separated two solutions of a univalent strong electrolyte the e.m.f. developed was shown to approach very closely that given by the equation

$$E = \frac{RT}{F} \ln \frac{C_1}{C_2}$$

where (C_1) and (C_2) represent the activities of the cation on the opposite sides of the membrane.

By an approximate mathematical development Michaelis showed (15) that this result may be derived from the more general equation for a diffusion potential

$$E = \frac{RT}{F} \frac{\frac{u}{n_c} + \frac{v}{n_a}}{u+v} \ln \frac{C_1}{C_2}$$

by assuming that the mobility of the anion v is equal to 0. Here (C_1) and (C_2) represent the activities of the salt on the two sides of the membrane, and, for a uni-univalent electrolyte $n_c = n_a = 1$.

Michaelis was led to suggest that the "dried celloidin membrane" is penetrated by pores within which there arises a quasi-equilibrium state in which there is for a long time a nearly constant diffusion gradient of the cation. The anion was supposed to be prevented from passing through the pores because of the interaction between its charge and that on the collodion. Another deduction from this work which is important from our point of view was suggested by consideration of the so-called "chemical" electromotive forces arising across membranes separating equally concentrated solutions of two different salts. Permeability to the anion being negligible the e.m.f. appeared to arise from the difference in mobility of different cations within the pores. Taking the mobility of the potassium ion as 1.00 it was possible to assign values to the mobilities of other univalent cations. These values are given in Table I, together with similarly compared values of the mobilities of the same ions in free diffusion in dilute aqueous solution. The mobilities are seen to be in the same order in the two cases, but with the differences greatly exaggerated in the first case, namely, that of the cations within the membrane.

The comparative mobilities of different cations in dilute aqueous solution, and within the dried celloidin membrane as calculated from the observed "chemical" electromotive forces are shown in Table I. (The mobility of potassium is in each case taken as one.)

TABLE I

Ion	Li	Na	K	Rb	H
Mobility in membrane.....	0.048	0.145	1.00	2.80	42.5
Mobility in dilute solution.....	0.520	0.650	1.00	1.04	4.9

From its bearing on the theory of these membranes it is important that both Fujita (9) working with Michaelis, and Collander (6), working independently, showed that non-ionized substances permeate the dried collodion membranes at rates which are inverse functions of their molecular volumes, the penetrability decreasing faster than the molecular volume increases.

Since in the many respects described above, the dried collodion membranes show permeability like that of living cells, it would be most desirable to ascertain how this selective semi-permeability is produced. In the case of the ordinary "wet" collodion membranes, Duclaux and Errera (8) showed that the relative rate of flow of various liquids through the membranes was determined by the viscosity of the liquids. This indicates

that the liquids flow through capillary spaces in the collodion. Hitchcock (10) and Bjerrum and Manegold (2) were able to calculate the approximate effective diameter of these pores, as shown by their permeability to water, to be about 10^{-6} cm. This is about one hundred times the size of the molecule of water in water vapor (23) or perhaps thirty to fifty times as large as the mean size of the aggregates of water molecules in liquid water at room temperature. It is obvious that membranes materially denser than the wet collodion membranes would begin to exclude the larger aggregates, and be less permeable to water than would be calculated from the diameter of their pores if this could be known. It is therefore not surprising that Northrup (18, 19) in his critical study of dried collodion membranes, which are far less permeable than "wet" membranes, found that the application of the method used by Hitchcock, and by Bjerrum and Manegold, led him to the result that the diameter of the pores in these membranes was of the order of 10^{-9} cm. Northrup points out that this result is obviously incorrect, and its incorrectness is still more apparent when we note that it leads to the conclusion that water, to which these membranes are permeable, passes through pores about one-tenth of the size of its own molecules. It is certain that any interpretation of the behavior of such membranes on the basis of the observed behavior of microscopic or even macroscopic systems is bound to be misleading. The pores, if they can be said to exist at all, are apparently smaller than the collodion molecules or at least not appreciably larger. This follows from the X-ray diffraction spectrum studies of cellulose by Sponsler and Dore (24), in which it has been shown that the native cellulose of ramie fiber consists of hexose rings in parallel chains slightly more than 1×10^{-7} cm. apart. Making due allowance for acetylation, we may estimate the thickness of the space occupied by these chains in collodion (cellulose triacetate) as about 1.5×10^{-7} cm. In all probability the chains are in collodion considerably broken up and no longer parallel. Under these conditions any material lying between these cellulose triacetate chains would be subject to strong intermolecular forces either in the form of molecular or ionic combining forces, electrostatic fields, or other less well understood forces.

Under these conditions the distinction between solution of a substance in the collodion and its penetration into "pores" in the collodion appears to break down. Until we know more exactly the intermolecular forces between collodion molecules and those of gases, water, or solutes, it will certainly be unsafe, and probably useless, to try to compare the collodion membrane to either a solvent or a filter as we ordinarily think of these. It may be added, however, that the relatively rigid collodion gel can with difficulty be related to ordinary liquid solvents whose molecules continuously change their positions and orientations relative to each other and to the solute molecules.

Northrup (18, 19) has pointed out that gases (H_2 , O_2 , N_2 , CO_2) pass through dried collodion membranes at rates unrelated to their density, and that H_2 and CO_2 pass with equal rapidity through such membranes whether or not the membranes are imbibed with water. Since such gases diffuse about 10,000 times more rapidly in a gaseous phase than in water, and since the pores of a well-wetted porous membrane are usually thought of as filled with water, he concludes that in the case of the dried collodion membrane the gas does not flow through pores. From what has been said above, it seems doubtful whether this distinction can have any real meaning in the present state of our knowledge. The experimental facts should, however, be carefully weighed in future attempts to formulate a theory of the mechanism of semi-permeability of such membranes.

Certain adsorption relations should also be borne in mind. Weiser (25) has shown that the permeability of copper ferrocyanide membranes may be explained on the basis of adsorption in the following way: "A distinctly porous membrane will be semi-permeable under two conditions: (1) if the membrane exhibits such marked negative adsorption for the solute that the pore walls are covered with a film of pure solvent which completely fills the pores; and (2) if the positive adsorption of the solute is sufficiently great to saturate the pores within the range that adsorption is practically irreversible." These conclusions are supported by parallel studies of the permeability of copper ferrocyanide membranes and of the adsorption properties of the copper ferrocyanide gel. It seems possible that this concept may be applied to differential adsorption of ions and molecules by collodion in relation to the permeability of collodion membranes. It is to be noted, for example, that phenol, which lowers the surface tension of water, passes in aqueous solution very rapidly through such membranes, while salts, which increase the surface tension of water and are therefore negatively adsorbed at a water-air interface, pass through very slowly. Michaelis' assumption that the negative charge assumed by collodion in contact with water repels the negatively charged anions is not incompatible with this idea, and both mechanisms may well be operative.

From the foregoing and other lines of evidence we may sum up our present knowledge of the structure of dried collodion membranes by saying that they appear to consist of a relatively rigid, though still elastic or sponge-like-mass of cellulose triacetate chains separated or interpenetrated by spaces not much, if at all, larger than the diameter of the cellulose triacetate chains themselves. In these spaces a large part of the molecules (water, gas, or solute) are more or less controlled in their movement by electrostatic forces, molecular bonding, or other forces loosely capable of inclusion under the term "adsorption." From such control arises the characteristic semi-permeability.

If in the case of pure collodion the membrane substance assumes a

negative charge against water, as may be supposed on the basis of its dielectric constant and its electrokinetic behavior, then a membrane of similar structure, but positively charged, should differ from a pure collodion membrane in being impermeable to cations rather than to anions. Acting on this prediction, Mond and Hoffmann (17) succeeded in making a positively charged membrane by mixing the basic dye Rhodamine B with their collodion. It is presumably bound to the collodion in some way which does not interfere with its basic dissociation, to which the positivity of the membrane may be attributed. Membranes so formed are permeable to anions but not to cations and in general behave as predicted, although with certain puzzling and probably significant quantitative differences. Höber and Hoffmann (12) made membranes having areas of pure collodion and of Rhodamine B collodion and showed that their electromotive behavior was predictable from those of the individual types of membranes separately.

The physiological significance of these experiments on artificial membranes is apparent upon comparison of their characteristics with those of living cells. This is strikingly illustrated in two papers; one by Mond, and one by R. Höber and J. Höber. The well-established notion that erythrocytes are permeable to anions but not to cations led Mond (16) to consider that their semi-permeability resulted from the presence of a positively charged membrane analogous to the Rhodamine B- collodion membrane. If this membrane were ampholytic in nature it should be possible by a change in pH of the surrounding solution to confer on it a negative charge and make it permeable to cations but not to anions. This change in permeability was actually observed to occur when erythrocytes were placed in an appropriate solution whose pH was more than about 8.0 to 8.3. This pH is slightly on the alkaline side of the isoelectric point of the globins of the erythrocyte, which may therefore form the actual anion permeable portion of the plasma membrane of normal erythrocytes.

Höber and Höber (11) studied the permeability of cells of the coenocytic marine green algae, *Valonia utricularis* (Roth) Ag. Actual penetration of substances through the cell wall and protoplasmic layer into the large vacuole can in this and similar species be determined by chemical analysis of the sap. It was found that cells placed in a glucose solution isotonic with sea water gave up no electrolytes at all. This is precisely what would happen if a dried collodion membrane separated *Valonia* sap from a glucose solution. Furthermore they found that cells placed in solutions rich in Br^- rather quickly took in that ion and lost Cl^- in exchange, whereas no exchange of cations could be detected unless the cells were somewhat injured by caffeine, whereupon K^+ was given up and Na^+ taken in by exchange. The cell was therefore permeable to Cl^- and Br^- , but normally almost impermeable to either or both K^+ or Na^+ . Since

analyses (14, 26) show the vacuoles of normal *Valonia* cells to contain a large proportion of K^+ , relatively little Na^+ , and almost no other cations, it is apparent that it must be slightly permeable, at some time at least, to both ions, and apparently more permeable to K^+ than to Na^+ . This corresponds to the relatively greater mobility of K^+ in aqueous solution and in collodion membranes referred to above. It is significant that injury by caffeine is necessary to make the cell perceptibly permeable to Na^+ , which because of greater hydration seems to have a larger effective radius than K^+ . Höber and Höber point out that the experimental results may be accounted for by assuming the protoplasm of *Valonia* to include a mosaic of two types of areas impermeable, respectively, to cations and to anions.

On the basis of this suggestion it has been possible to account on a strictly physico-chemical basis for many if not all the puzzling phenomena of selective absorption and accumulation of ions by plant cells (4, 5).

In the case of *Valonia*, for example, H^+ and HCO_3^- are present in the sap in much higher concentration than in sea water. The H^+ concentration is at least 100 times that in sea water, and the H^+ will tend to diffuse out of the cell through the postulated cation permeable areas, which may be of any dimensions large enough to exceed in size the effective range of inter-ionic electrical forces. Since anions cannot accompany the H ions through these areas, electrical neutrality can be maintained only if a cation enters in exchange for each hydrogen ion which leaves the cell. Theoretically this exchange can go on until the concentrations of H^+ and the other cation in the sap and in the sea water are such that the work yielded by the outward diffusion of a hydrogen ion is equal to the work which must be expended to make the other cation pass into the cell. This condition may be expressed for the transfer of any small amount, dn , of H^+ and of any other cation, e.g., K^+ , by putting:

$$dn \frac{RT}{F} \ln \frac{(H^+)_1}{(H^+)_2} - dn \frac{RT}{F} \ln \frac{(K^+)_1}{(K^+)_2} = 0$$

Therefore at equilibrium

$$\frac{(H^+)_1}{(H^+)_2} = \frac{(K^+)_1}{(K^+)_2}$$

In other words the "ratio of accumulation" of K^+ may approach but not exceed the ratio of the H^+ concentrations in the sap and the sea water.

Netter (18) has given a very elegant exposition of this theoretical basis and has shown that the predicted distribution ratios are approached in a system of the initial constitution:

K_2SO_4 0.000715 M		anion impermeable		K_2SO_4 0.000715 M
H_2SO_4 0.1 M		collodion membrane		Glucose 0.213 M

as well as for other similar systems. His paper also includes significant applications of these findings to the cases of erythrocytes and muscle cells. Netter, however, assumes that living cells are constantly in equilibrium with the surrounding solution, or very close to it, in so far as concerns ions to which they are permeable.

The actual K^+ concentration normal in the sap of the different species of *Valonia* is about 0.5 M, while that in sea water is about 0.01 M. The ratio of accumulation for K^+ is therefore about 50, while the concentration ratio for H^+ is more than 100. The ratios of accumulations for other cations are not only less than that for K^+ , but actually less than one. It seems, therefore, that accumulation of K^+ has been accomplished, but that equilibrium has not been reached, because otherwise the remaining cations would have accumulation ratios equal to that for potassium.

A similar train of reasoning explains the exchange of HCO_3^- for Cl^- and the slight accumulation of Cl^- (roughly 0.6 M in the sap, 0.5 M in sea water).

Since there is constant production of H and HCO_3 ions by respiratory metabolism, and they are constantly replaced by other ions from sea water, the amount of osmotically active material in the cell increases continually. Under these conditions the cell will take in water and grow by increase in cell size. This has been suggested by Osterhout (21) in connection with a different hypothesis as to the mechanism of accumulation.

The discrepancy between the ratios of accumulation for different cations may be explained along lines suggested by André and Demoussy (1). They first pointed out that so long as equilibrium was not attained in any process of diffusion of ions, the more mobile ions would always be present in more nearly the equilibrium concentration. The distribution of K^+ and Na^+ in sugar beets was found to be in conformity with this idea. In *Valonia* it is possible to show by direct experiment that a non-equilibrium state exists normally (4, 5). The experiments were done with *Valonia macrophysa* Kütz. Cells were placed in sea water and in various mixtures of sea water with isotonic KCl or NaCl solutions, thus increasing or decreasing the ratio of K to Na in the solution bathing the cells. If equilibrium existed, then the ratio of K to Na in the sap should have changed in the same sense in which it had been changed in the surrounding solution. This was not the case. The ratio of K to Na in the sap always increased, though very slowly, regardless of whether the similar ratio in the solution bathing the cells was greater than normal or less. Since the relatively high concentration of H^+ within the cell provides the necessary condition for accumulation of cations, K^+ and Na^+ will tend to enter the cell. Both on the basis of theoretical expectation, and of experiments with other species of *Valonia*, K^+ seems to be the more mobile and therefore it is to be expected that its concentration will approach nearer to the theoretical limit than will

that of Na^+ . As a matter of fact, water is drawn in so rapidly to keep pace with the entry of K^+ and Cl^- that it may enter even faster than Na^+ , which then is present in lower concentration inside the cell than outside.

The great difference in the penetrability of K^+ and Na^+ in this case is like that encountered in artificial membranes, but appears to be even more pronounced.

The increase in the ratio of K to Na in the sap of *Valonia* in the experiments just mentioned can best be explained by supposing that the permeability to K^+ is increased by any change in the ratio of K to Na in the surrounding solution, and that it is increased more than is the permeability to Na^+ . This supposition follows naturally from the idea that the protoplasm of *Valonia* acts like a porous collodion membrane through whose pores molecules or ions can pass, provided the pores are large enough. Presumably the penetrability of a given molecule or ion varies with pore size, being completely suppressed in pores of less than a certain diameter, then increasing with increasing pore diameter at first slowly, then more rapidly and finally approaching as a limit the mobility of that molecule or ion in dilute aqueous solution. In general the larger the molecule the larger must be the pore for penetrability to become apparent. This idea is given qualitative support, in the case of molecules, by the work of Fujita (9) and of Collander (6) on the permeability of dried collodion membranes. Figure 1 shows what might reasonably be assumed

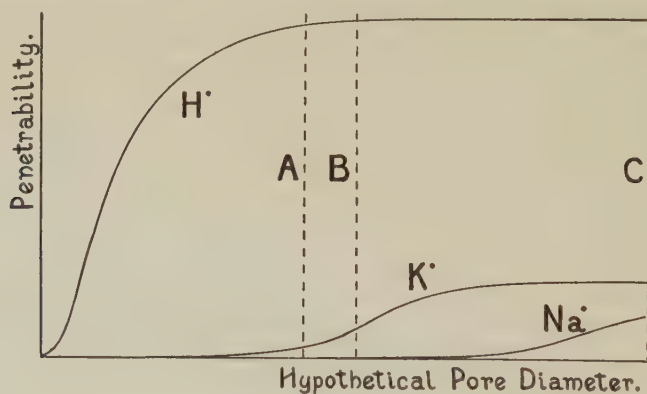


FIG. 1

to be the relations in the case of the three cations: H^+ , K^+ , and Na^+ . In this figure penetrability is plotted as ordinates and hypothetical pore diameter as abscissas. The penetrabilities approach values which are in the same ratio as the mobilities of these ions in dilute aqueous solution, and they appear at relative pore diameters proportional to ionic diameters calculated from the corresponding mobilities in dilute aqueous solution.

Presumably the increase in penetrability with increase in pore diameter would in actual cases appear as a statistical result of the increase in number of pores of adequate size, as well as of the increase in diameter of individual pores.

This explanation has been given in terms of pores for the sake of simplicity; but it will be seen that any mechanism sufficing to explain the characteristics of collodion membranes may be applied to living cells just as well. It is also quite possible that inside some cells H^+ and HCO_3^- are not present in concentrations great enough to supply the motive power for the observed ion accumulation; but any diffusible ions produced inside the cell will answer equally well: weak or strong acids, or bases, or their salts, would be equally effective.

The hypotheses just outlined make possible many interesting correlations between observed phenomena of cellular oxidations, growth by increase in cell size, and ion accumulation in plant and animal cells. It must not be forgotten, however, that ever since the classical experiments of Overton (21) it has seemed unavoidable to conclude with him that fat-like materials (including lipins and sterols) are present, probably as an emulsion, in the plasma membrane. To this concept we may now add the further concept of a mosaic of anion and cation permeable regions consisting, in all probability, of protein gels either pure or mixed with other water soluble substances.

SUMMARY

Selective ion accumulation by living cells may be explained if we take into account the following necessary conditions:

1. The existence of a normal state of non-equilibrium between living cells and their environing solution. This has been shown to be the actual state in the investigated cases.

2. The presence in the protoplasm or in its surfaces of a mosaic of two kinds of areas which are impermeable to anions and impermeable to cations, respectively, thus resembling the artificial models which have been prepared, and which show a type of permeability to a large variety of ions and molecules, strikingly like that of living cells, and are experimentally capable of leading to the movement of ions against their concentration gradients.

In all probability both living protoplasm and the analogous artificial membranes possess a structure so minute that the question as to whether their permeability is due to solvent power or to pore diameter becomes to a considerable extent artificial. In many respects it is easier to visualize the mechanism of selective permeability in terms of pores.

Changes in permeability to ions may also be visualized as due to

changes in pore diameter, and in the sign and density of the charge on the pore walls. Such changes probably affect the penetrability of different ions in differing degrees varying with the physical and chemical structure of the membrane.

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IX. OBSERVATIONS ON DUNALIELLA VIRIDIS TEODORESCO

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1. *Statement of the problem.*—In highly concentrated salt solutions, which form a problem of study in this laboratory, there occur a number of green flagellates which are of truly cosmopolitan occurrence wherever salt is made and wherever natural waters become concentrated. One of these, *Dunaliella viridis* Teodoresco, has been studied by Teodoresco (22) and Peirce (19), both of whom have contributed valuable data to our knowledge of life under unusual conditions.

The following table shows the composition of certain natural brines in which this form occurs.

Substance	Searles Lake, California	Owens Lake, California	San Francisco Bay
MgCl ₂			8.17
KCl	4.69	3.74	2.57
NaCl	16.63	17.44	17.43
Na ₂ SO ₄	6.98	5.40	5.04
Na ₂ CO ₃	3.46	5.58	
NaHCO ₃	.77	1.43	.08
Na ₂ B ₂ O ₄	1.39		
Na ₂ B ₄ O ₇	.43	2.72	.15
(pH)	9.48	9.62	6.90)
H ₂ O	65.65	63.69	66.56

The natural environment is liable to sudden and drastic changes, both in concentration and temperature (Peirce, 19, and von Kallezinsky, 11). Also, from the point of view of osmotic pressure, as well as that of ionic antagonism, there appears to be no reason why such organisms should exist; that they do exist proves either that the organism is utterly unusual or that the rules derived by and for organisms living in fresh water or sea water are inapplicable to these beings. The latter assumption appears to be worthy of further consideration chiefly because a survey (to be published elsewhere) has shown that at least fifty organisms, both plant and animal, belonging to the most widely divergent classes, are capable of leading a normal existence in solutions which contain more than 20 per cent of salts.

The consideration of *Dunaliella* has led to certain generalizations. That

these generalizations have to be taken with more than "a grain of salt," will become apparent soon enough.

Several strains of *Dunaliella viridis* were obtained from widely separated localities such as Setubal, Portugal; Lake Tekir Ghiol, Roumania; the lagoon of Araruama, Brazil; Lake Cumana, Venezuela; Soda Lake, Nevada; Sand Springs, Nevada; Great Salt Lake, Utah; Owens Lake, California; Searles Lake, California; Marina, California; and Leslie Salt Works, San Mateo, California. The latter three strains were used in these particular investigations.

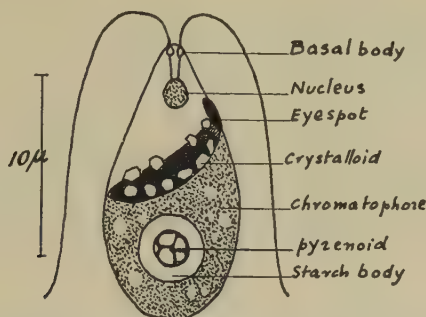


FIG. 1.—Diagrammatic sketch of *Dunaliella viridis* Teodoresco. Nucleus and basal bodies visible only after staining, e.g., with neutral red.

In all cases we have a naked, green bi-flagellate ($12 \times 8\mu$), with a cup-shaped chromatophore and a red eye-spot. There are two basal bodies and a nucleus in the colorless anterior portion. In the posterior part we find a doughnut-shaped starch body and a pyrenoid, which is often situated in the center of the "doughnut."

The swimmers are exceedingly plastic. Under adverse conditions they may encyst either before conjugation (vegetative cysts) or after conjugation of the isogametes.

There is very little difference in the morphology of the strains isolated from various localities. As will be seen later, however, there are certain physiological differences between the strains.

As preliminary culture experiments by Dr. C. B. van Niel showed, the organisms developed best in a medium of the following composition:

Water	100
NaCl	15
NaHCO ₃	.1
K ₂ HPO ₄	.02
MgCl ₂	.02
KNO ₃	.02
Adjusted by K ₂ HPO ₄ to a pH of about 9	

The cultures developed aërobically in continuous light at approximately 22°C. It is possible, however, to vary the pH between 6 and 9 or to increase the salt content to 5.25 M NaCl. In both cases the time required for development is greatly increased.

2. *Influence of concentration* (Mr. Ralph Hawkins collaborating).—Whenever we wanted to change the culture solution, the culture fluid containing the organisms was placed in a shallow dish under directed light. Within thirty minutes the organisms had accumulated at the end of the dish away from the light source. They were then taken out by means of a capillary pipette and placed in the solution to be investigated. A culture was developed in concentrated NaCl solution (5.25 M) at pH 7. This culture was mixed with varying amounts of water, the number of active swimmers was observed, and then it was placed in a sealed chamber. Survival counts were made after 1, 2, 4, and 12 hours, and the drop then transferred to an Abbé refractometer. From the refractive index at 25°C. the final concentration of salt could be ascertained.

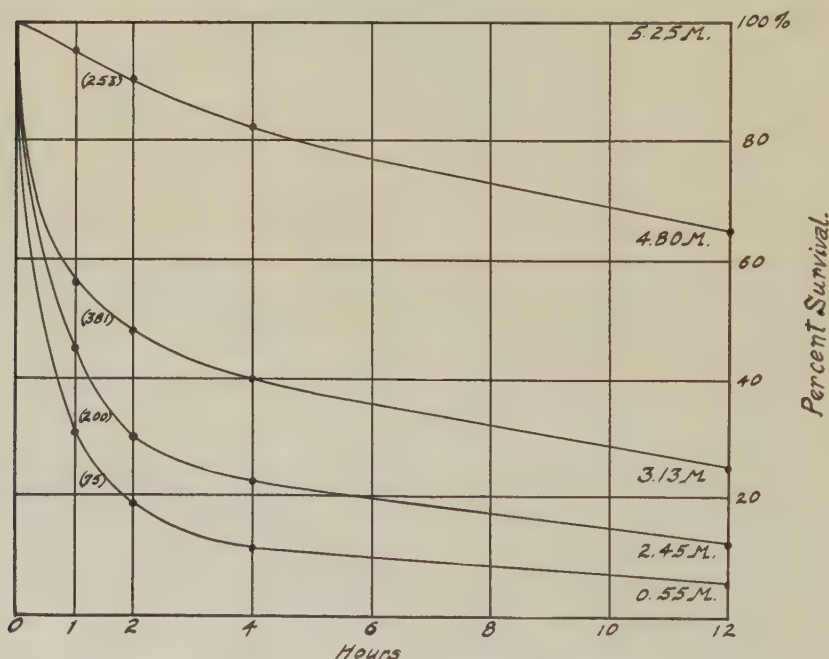


FIG. 2.—Percentage survival of *Dunaliella* when transferred from 5.25 M NaCl to lower concentrations indicated in the figure. The numbers in parentheses refer to numbers of organisms in each experiment.

In Figure 2 the percentage survival is shown. It is apparent that below 2.5 M NaCl the damage is pronounced, regardless of dilution. As

we did not succeed in preparing dilutions below .5 M, we have to suspend judgment on these data until they are more complete. They illustrate, however, that sudden dilutions need not be fatal to *Dunaliella*. Sudden concentrations of the liquid (excluding the action of crystallization as described by Peirce, 19) do not result in 100 per cent mortality either (see accompanying figure). It might be added that the Searles, Marina, and Leslie strains showed similar behavior, but that the Searles strain was in all respects the hardiest.

3. *Hydrogen-ion concentration*.—Although the optimum development

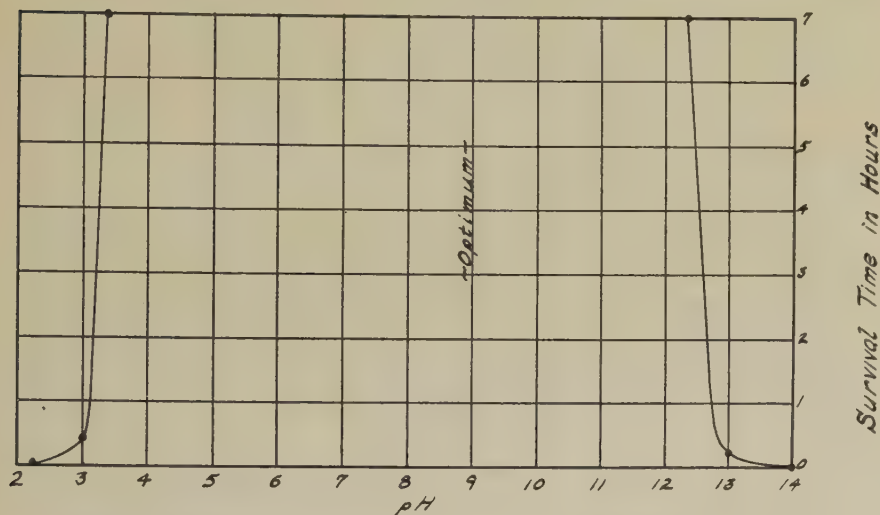


FIG. 3.—Influence of H-ion concentration on *Dunaliella* in 5.25 M NaCl.

takes place at pH 9, the organisms are able, with impunity, to stand a pH as acid as 4 or as alkaline as 11 for 24 hours. The accompanying diagram shows survivals of the Searles strain in 5.25 M NaCl buffered to various pH. At the extreme values of acidity and alkalinity the organisms, while still active, become spherical. The survival time of Searles strain of *Dunaliella* in 5.25 M NaCl buffered to various pH values is given in Table I.

TABLE I

Survival Time	pH
12 minutes	13
7 hours	12.25
24 hours	4
24 minutes	3
2 minutes	2.25

4. *Temperature*.—Teodoresco found the thermal death-point of *Dunaliella* to be about 50°C., while the cysts were able to survive up to about 59°C. We have been able to confirm this statement for all strains except a single one from Searles Lake, which, together with the other organisms in this brine (blue-green algae, purple spirilla, and protozoa), was able to withstand in active vegetative condition temperatures of considerably over 90°C. for several hours. The Walton hot-stage used in these experiments was calibrated carefully by means of the substances of known melting-points shown in Table II.

TABLE II

Substance	M P in °C.
Benzoic anhydride	42.0
D. menthol	43.0
Trichloroacetic acid	57.3
Coumarin	67.0
Vanillin	80.0

The values were confirmed for five series. The thermal death-point proved to be independent of concentration in the range 2.5 M–5.25 M NaCl and pH 6–11 (D. Straup collaborating). Because all organisms in the Searles Lake brine seemed to be thermotolerant, it appears probable that some factor in the external environment predisposes toward extreme thermotolerance. The tentative explanation which we have to offer for

TABLE III

SURVIVAL OF BRINE ORGANISMS AS AFFECTED BY VARIOUS ANIONS

No.	Substance	Molarity	Concentration in weight, percentage	Survival time	Remarks
1	KI	2.5	41.5	∞	Phototaxis normal
2	KBr	2.5	30.0	∞	Phototaxis normal
3	KCN	2.5	16.28	∞	<i>D. viridis</i> only*
4	NaCNS	2.5	20.27	∞	Phototaxis normal
5	NaNO ₃	2.5	21.25	∞	Phototaxis normal
6	Na(HCOO) ..	2.5	17.00	∞	Phototaxis normal
7	K ₂ C ₂ O ₇	(in 2.5 M NaCl)	5.00	∞	Phototaxis normal
8	K ₂ Cr ₂ O ₄	2.5	48.55	∞	Phototaxis normal
9	NaBO ₂	(in 2.5 M NaCl)	1.39	∞	Phototaxis normal
10	Na ₂ B ₂ O ₇	(in 2.5 M NaCl)	.43	∞	Phototaxis normal
11	KMnO ₄	(in 2.5 M NaCl)	2.5	0	Instant death, stains brown in toto, shrinks.

* Caudate *Dunaliella* and *Trichomastix* die in 35 minutes.

∞ This symbol means that the organisms were still alive after 6 hours.

this extraordinary behavior will follow logically from our final considerations.

The observations of Teodoresco (22) on the tolerance of low temperatures by *Dunaliella* (to -35°C . in the vegetative state) have also been confirmed.

5. *The influence of anions.*—For these and the following experiments a culture from Marina (2.5 M NaCl, pH 9) was chosen because it contained, besides *Dunaliella viridis*, a typical caudate *Dunaliella* and a colorless flagellate closely resembling *Trichomastix salina* Entz, previously described by Geza Entz, Sr., from salines in Hungary (4). It was felt that observations on various organisms, as in the case of thermotolerance, might lead to more reliable generalizations. It proved that, in principle, none of the organisms (with a few exceptions) were materially affected by anions.

The very feeble toxic effect of 2.5 M (16 per cent) KCN may be ascribed to hydrolysis. For the dissociation constant of HCN is only

$$7.2 \times 10^{-10}. \text{ We have, therefore, } \frac{K_{\text{water}}}{K_{\text{acid}}} = K_{\text{hydrolysis}} = \frac{(\text{OH}^-)^2}{(\text{salt})}$$

$$\text{or, approximately, } \frac{10^{-14}}{7 \times 10^{-10}} = \frac{x^2}{2.5}. \text{ Therefore the pH} = 14 - \log x =$$

11.8, which may easily be toxic for *Trichomastix* and for the caudate *Dunaliella*! Similarly, sodium formate was non-toxic. The dissociation constant of formic acid is 2×10^{-4} , corresponding to a pH = 8.5 for 2.5 M sodium formate, a value well within the non-toxic range.

The definite chemical action of the permanganate ion may account for its toxicity.

6. *The influence of cations.*—In order to exclude the effects of hydrolysis, the hydrogen-ion concentration was determined in the solutions of all heavy metals and values for the death time were considered only when the pH was not less than 4.

Figure 4 (p. 108) shows graphically the influence of ammonium ion and of bivalent and trivalent cations on *Dunaliella*. The damage caused was always similar. The organism seemed to be glued to the slide by the ends of the cilia, rocking to and fro. This is shown in Figure 5 (p. 109). Whenever the body came in contact with the slide it stuck there and began to shrink. Finally the protoplasm disintegrated into a fine granular mass.

Of the monovalent alkali metals, Na, K, and Li were non-toxic in 2.5 M solution. Both 2.5 M $(\text{NH}_4)_2\text{SO}_4$ and 2.5 M NH_4Cl killed in 12 to 15 minutes. The actual penetration of NH_4 ion could be demonstrated by the method used by Chambers (3) for starfish eggs. *Dunaliella* was stained with neutral red; the basal bodies, pyrenoid, nucleus, and several other granules stained a deep red, showing that their pH must be neutral

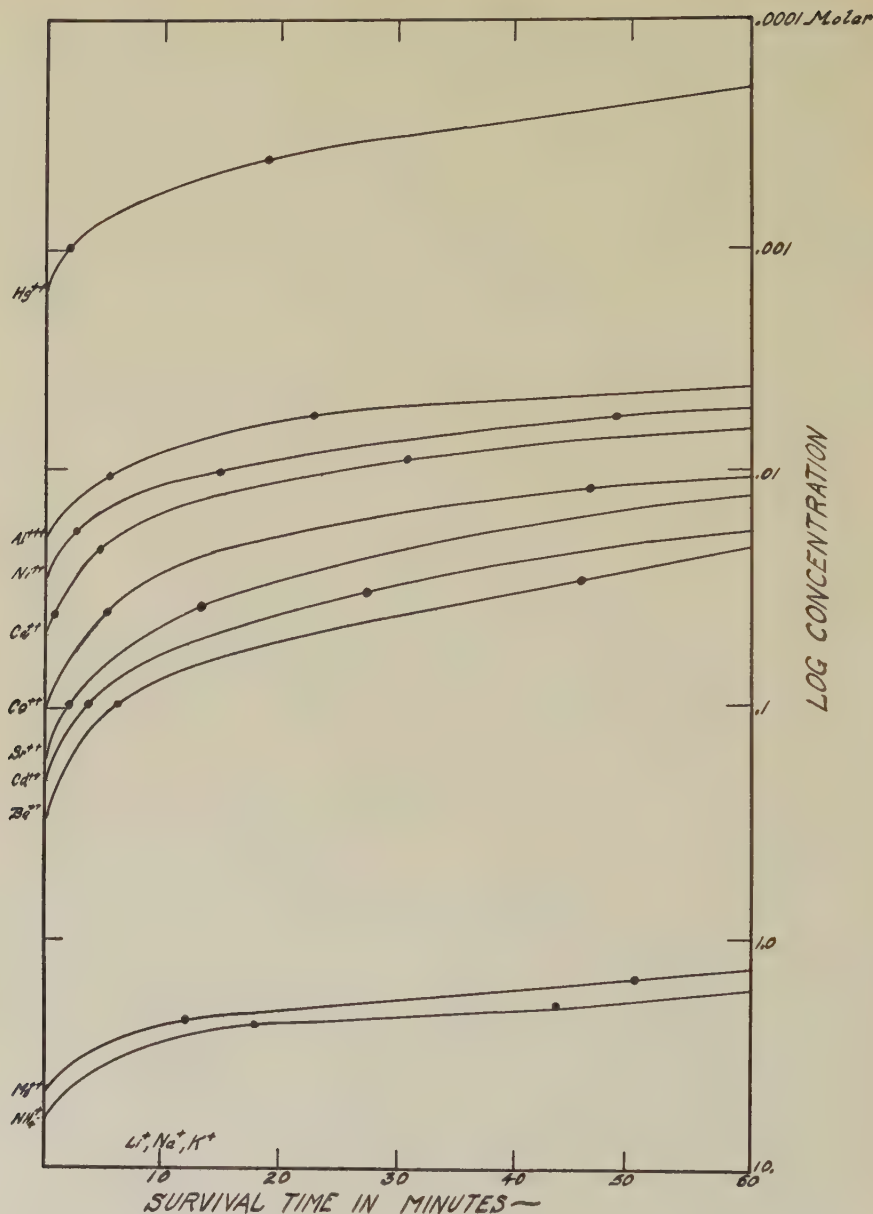


FIG. 4.—Survival of *Dunaliella* in various cation concentrations.

or acid. (Experiments on the penetration of dyes are now under way.) Application of 2.5 M NH_4Cl caused, within 3 minutes, a change in this color to yellowish orange or lemon yellow. After the organism began to

shrink the red color returned to the granules before they finally disintegrated.

MgCl_2 is only faintly toxic in 2.5 M solution. Its action is intermediate between univalent and bivalent ions, as is often observed in the lyotropic series.

According to these experiments, whenever a metal can be brought into a complex anionic state it should be non-toxic. A satisfactory confirmation of this conception seems to be provided in the following: Mercuric iodide, HgI_2 (M.W. = 454.4) is only very slightly soluble in 2.5 M KCl. A solution of 3 mgr. HgI_2 in 100 cc. 2.5 M KCl is therefore about 7×10^{-5} M. This solution proved to be highly toxic for the flagellates. HgI_2 ,

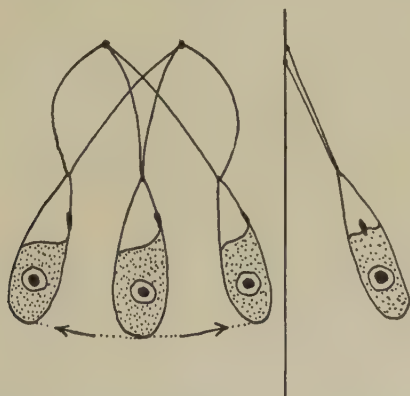


FIG. 5.—Diagrammatic sketch showing “ground plan and elevation” for *Dunaliella*, illustrating damage due to cations.

however, when dissolved in KI, reacts as follows: $\text{HgI}_2 + 2\text{KI} = \text{K}_2\text{HgI}_4$ in which the mercury is present in the anionic form, as HgI_4^- . A solution of HgI_2 in 2.5 M KI, in which the concentration of mercury was 1.1×10^{-3} M, was only faintly toxic to *Dunaliella* (survival time 45 minutes) notwithstanding the fact that the concentration of mercury was 100 times that of the preceding experiment.

Similarly $\text{Hg}(\text{NO}_3)_2$ in 2.5 M NaNO_3 is very little soluble but highly toxic even in a concentration of 10^{-5} M. However, HgCl_2 in 2.5 M NaCl , where the “common ion” effect operates (although with less strength than in the iodides), though very soluble, showed a lessened toxicity in a concentration of 10^{-3} M (survival time 4 minutes).

The foregoing experiment was suggested to me by my colleague, Dr. C. B. van Niel.

It appears from the table that cadmium shows much less toxicity than is required by its nature as a heavy metal. Since we “diluted” our cadmium solution with 2.5 M NaCl , we have to count on the presence of CdCl_2

of which Lewis and Randall (13) say: "...it cannot be regarded as a strong electrolyte. It possesses, in a lesser degree, the characteristics of an analogous substance, mercuric chloride, which shows almost no ionization." The results with cadmium are, therefore, well in keeping with the observed facts.

The exceptionally pronounced nature of the lyotropic series, the enhanced difference in action between the alkali metals, and the position of the heavy metals are outstanding. We could cite endless papers on these and similar questions. Let it suffice to point out that: (a) the lyotropic series exists; not valency alone determines the action of ions; (b) the action of ammonium and magnesium salts on the precipitation of egg-albumen is similar (Pauli, 18); (c) the values make it appear that the colloids concerned are lyophilic and are salted out.

It appears, therefore, that the cell membrane and the flagella have a strong negative charge, which can be neutralized by cations in a lyotropic series. According to Michaelis (15) and Fujita (7) the membrane should be impermeable to anions and selectively permeable to cations. The discharge of the particles causes the adhesion of the organisms to the negatively charged glass (Hardy, 9).

Egg-white, according to Mines (16), behaves similarly in that, even in a concentration of .0016 M, La^{+++} precipitates it, while monovalent cations are inactive in this respect.

7. *Hydration*.—Bungenberg de Jong (2), and also Kruyt (12), recognize two stabilization factors for lyophilic colloids, the first factor being charge, the second hydration. Lyophobic colloids possess only one stabilization factor, the electric charge. It should be possible, therefore, to dehydrate particles like those forming the surface of *Dunaliella* by means of various non-electrolytes, the dehydrated particle being very sensitive to electrolytes, then flocculating as if electrolyte had been added.

Kruyt and de Jong recognize two types of dehydration as exemplified by the action of various substances on a $\frac{1}{2}$ per cent agar sol: (a) dehydration as a result of mass-action, e.g., ethanol forming alcohol hydrates in the external phase; (b) dehydration by adsorption, e.g., tannin. In another paper de Jong (10) studies the effects of various phenols on the dehydration of agar and concludes that the action of phenols as dehydrating agents is proportional to the number of phenol groups in the molecules.

Na-salicylate is faintly toxic to *Dunaliella* in a 2.5 M solution (survival time 1 hour). This toxicity cannot be due to pH as salicylic acid is a fairly strong acid ($K_{\text{acid}} = 1.06 \times 10^{-3}$). The salicylate radical has one phenol group. Resorcinol is very toxic even in concentrations of .02 M. The organisms stick to the slide and shrink. Resorcinol has two

phenol groups. Tannic acid is definitely toxic in concentrations as low as .0002 M. It has 25 phenol groups.

Dehydration by mass-action is also very pronounced in *Dunaliella*. The action of ethyl alcohol is a case in point. A 2 M solution is definitely toxic (the organisms stick to the slide and shrink), but a 1.5 M solution of ethyl alcohol (=6.9 per cent by weight, 8 per cent by volume) shows only little "cation damage." When we compare our values for toxicity to *Dunaliella* with those of de Jong for the dehydration of $\frac{1}{2}$ per cent agar sol we get the result shown in Table IV.

TABLE IV

COMPARISON OF THE ACTION OF VARIOUS NON-ELECTROLYTES ON A $\frac{1}{2}$ PER CENT AGAR SOL AND ON DUNALIELLA

Substance	Concentrations Required to Dehydrate a $\frac{1}{2}$ Per Cent Agar Sol	Concentrations About Equally Toxic to <i>Dunaliella</i>
C ₂ H ₅ OH	40 per cent	8 per cent
Resorcinol	.1 Molar	.02 Molar
Tannic acid	.00059 Molar	<.01 Molar

The dehydration of a $\frac{1}{2}$ per cent agar sol requires a concentration of the agent 5 times that required for the organism studied. It seems, therefore, justified to assume that, in spite of the a priori improbability, the cells of *Dunaliella* behave like hydrophilic colloids. It may very well be that the abnormally high values obtained with the cations were due partly to the replacement of the monovalent cations on the membrane—a sort of ionic antagonism—although the true physiological antagonism is absent because the swelling and peptizing action of the monovalent cation is counteracted in the normal environment not primarily by the alkaline earths, but possibly by the dehydration effect of the strong salt solution. A second reason for the small cation effect may be depressed activity in high electrolyte concentration. This factor, however, must be small.

9. *Imbibition pressure*.—Carl Ludwig (14), in a classical experiment of eighty years ago, demonstrated that a thoroughly dried animal membrane was capable of extracting water from concentrated NaCl solution, causing this solution to deposit crystals. J. Reinke (21) showed, in 1879, that *Laminaria* under a pressure of 42 atmospheres was still able to take up 16 per cent of water. Dumanski, in 1907, found that gelatin is able to take up water from a concentrated K₂SO₄ solution. Bayliss (1) showed that gelatin could take up water from 90 per cent ethyl alcohol. It is only after the theoretical and experimental work of Posnyak (20), Freundlich (5), Polanyi, and Katz that we begin to see the import of this phenom-

enon; that we see why Overton (17) already in 1904 found that he could with imbibable colloids equalize very high osmotic values. For instance, bovine serum proved to be isotonic with .9 per cent NaCl. If this serum were made five times more concentrated it proved to be isotonic with 5 per cent NaCl; six times more concentrated serum was isotonic with 10 per cent NaCl.

Flusin (6) has shown that if a membrane is in contact on one side with a liquid *A* which causes swelling, on the other side with a liquid *B* which does not cause swelling in the same degree, liquid will pass through the membrane from *A* to *B*.

We still lack two logical links in our chain. One of these is provided by Polanyi, who has shown, on thermodynamic grounds, that positively adsorbed substances increase imbibition while negatively adsorbed substances decrease imbibition. The last link is given by Hofmeister, who showed that the action of neutral salts on imbibition follows the lyotropic series.

Inasmuch as the electrolyte in our culture medium could exert a maximal osmotic pressure of about 90 atmospheres (assuming an activity for 2.5 M NaCl of 1.620), the imbibition pressure has to exceed this amount. Imbibition pressure equals *X* minus osmotic pressure, in which *X* has to be a positive pressure, which keeps the organisms in shape. Posnyak (20) calls attention to the fact that this imbibition pressure decreases rapidly with increase in liquid content of the gel. As a matter of fact, *Dunaliella* is highly dehydrated. Van Leeuwen and I have measured the specific gravity of *Dunaliella* cysts at 20° and found it to be $1.16 \pm .02$. No Brownian movement could ever be demonstrated in *Dunaliella* cells.

In *Dunaliella*, both internal and external conditions seem to warrant the hypothesis that in this case an imbibition pressure would more than counterbalance the osmotic pressure of the external milieu.

10. *Temperature and coagulation.*—A great many authors have shown that increase in concentration in the external milieu leads to a higher lethal temperature (de Vries, 24). A very tempting analogue is offered by the coagulation of As_2S_3 sol, in which process the monovalent cations became *less* effective with increasing temperature, while the bivalent cations became *more* effective.

When we look again at the composition of the natural environment of *Dunaliella* we see an almost total absence of bivalent cations in Searles Lake, while the Mg is present in the brines of marine origin. It has been pointed out above that the Searles strain was the only one which possessed the very pronounced thermotolerance.

Culture experiments showed that: (a) the Searles strain used as infection material in media containing Mg gave rise to a strain of *Dunaliella* which had lost its thermotolerance (death-point 48°5C.); (b) Marina

and Leslie strains refuse to grow upon media deprived of Mg ion. This seems to indicate that we might possibly ascribe thermotolerance to the almost complete absence of bivalent ions from the medium!

11. *Summary and conclusions*.—(1) *Dunaliella viridis* must be looked upon as an organism the outer surface of which has lyophilic properties and a pronounced negative charge. (2) Imbibition pressure may account for its osmotic behavior. (3) The thermotolerance of certain strains may be due to the absence of calcium and magnesium from their environment.

The author is deeply indebted to Dr. H. L. van de Sande-Bakhuyzen, and to Dr. C. B. van Niel, whose suggestions have been of material assistance in the pursuit of this study.

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Freundlich's *Kapillarchemie* contains a great deal of information on imbibition pressure. It was deemed, therefore, superfluous to cite separate authors on this topic, particularly in connection with this very short paper.

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X. EXPERIMENTAL CHANGES IN THE PERMEABILITY OF THE CELL

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Of the many problems which are involved in the theory of permeability, that concerning the changes in permeability of the cell under experimental conditions and in different states is of fundamental interest. In the first place, we can learn from them the importance of permeability for function; in the second place, the fact that it is possible under experimental conditions to influence quantitatively the permeability of the cell to different substances creates the possibility of solving important problems of experimental medicine. It would be, for instance, of greatest importance if we could so influence the absorption of drugs that effects of long duration and constant intensity would be obtained.

I shall give some examples in this paper showing the change in permeability of the frog's muscle under experimental conditions. These investigations are typical examples which show the influence of external factors on permeability. On the other hand, activity of the cells leads to a change in permeability and it will be of interest to show in what way this interferes with changes in permeability caused by external conditions. Further we shall discuss the influence on the cell of internal secretions and poisons of the autonomic system with the purpose of clarifying the rôle of internal secretions and that of the autonomic innervation on permeability in living animals.

From the osmometric experiments of Overton (10, 11, 12) it is known that KCl enters the cells of the striated muscle. So it is very probable that the KCl contracture is due to the penetration of KCl. If this is true it is to be expected that the size of the KCl contracture can be influenced by a change in the ion-composition of the liquid, since we know that in this manner we can modify the permeability of the cell. In preliminary experiments it was found that the KCl contracture was completely reversible provided that the concentrations of KCl in the intervals between the individual experiments had been chosen correctly. This furnished the basis for a study of the effects of different solutions on the susceptibility of the muscle to KCl. First the influence of the alkali cations was studied. It was found that if a small part of the Ringer solution was replaced by corresponding quantities of isotonic solutions of alkali chlorides the KCl contracture increases according to the series $\text{Li} < \text{Na}$, $\text{Cs} < \text{NH}_4 < \text{Rb}$. At the same time we observe that the latent period of the contracture is diminished if the contracture is increased. It is of interest that this series corresponds to the imbibition series in muscle as it was found in a paper

(4) in 1923. We can say that the KCl contracture is increased in the same order that the tendency of the solution to increase imbibition of the muscle increases. This illustrates for the muscle in a rather simple way the fact found by different investigators of plant and animal cells, that frequently the permeability of a cell is dependent on its imbibition (5).

Because we know that in general the cations of the alkali earths decrease imbibition and permeability, it was to be expected that decreased KCl contracture would be observed in the presence of these salts. In fact, as Figure 1 shows, the KCl contracture is suppressed by these salts so

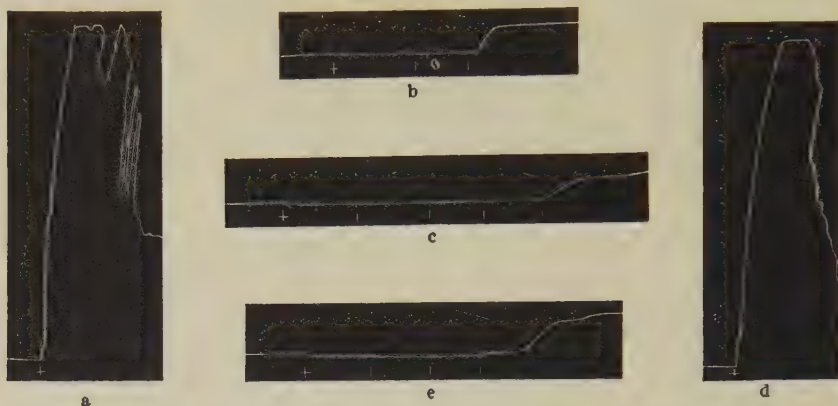


FIG. 1.—Sartorius muscle (*R. temporaria*). At +, 2 cc. isotonic KCl solution is regularly given; at every sign like 1 (in Fig. 1, *b*, *c*, and *e*) 1 cc. KCl was added. The composition of the solutions in which the muscle was immersed was as follows:

a { 14 cc. Ringer solution
1 cc. isotonic NaCl solution

b { 14 cc. Ringer solution
1 cc. isotonic CaCl_2 solution

c { 14 cc. Ringer solution
1 cc. isotonic SrCl_2 solution

d { 14 cc. Ringer solution
1 cc. isotonic NaCl solution

e { 14 cc. Ringer solution
1 cc. MgCl_2 solution

strongly that we must increase the concentration of KCl many times to obtain even a trace of a contracture. These experiments made it seem probable that the different effects of a pure NaCl solution and a balanced Ringer solution could also be demonstrated by the KCl contracture. This is proved in Figure 2. In this experiment the KCl concentration which we used was ineffective after Ringer solution. However, if we replaced a part of the Ringer solution with isotonic NaCl solution the same KCl concentration which was previously without effect now produced a contracture, which was larger the more the Ringer solution was replaced by NaCl solution. Further experiments showed that the heavy metal ions, Co^{++} , Cd^{++} , and Fe^{+++} also are able to diminish or to suppress completely

the KCl contracture. They had the characteristic influence on the KCl contracture in even smaller molecular concentrations than the alkali earths. From other experiments it is known that the H-ion concentration also influences the permeability. For instance, Osterhout (9) observed that permeability in plant cells is diminished in acid solution and is increased in alkaline solution. The same holds for the KCl contracture.

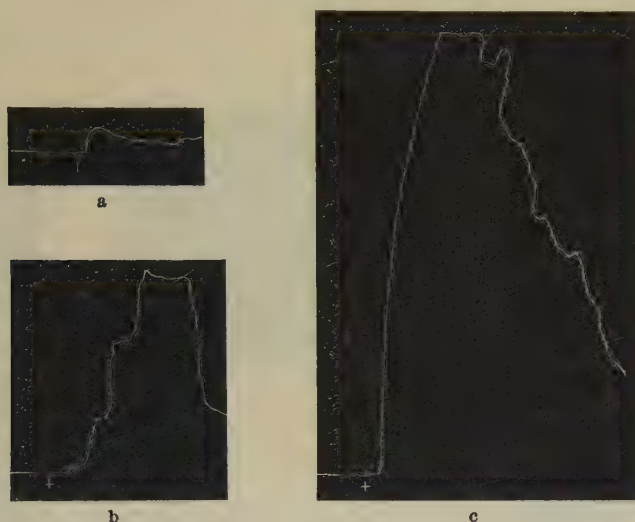


FIG. 2.—Sartorius muscle. At +, 1 cc. KCl solution is added.

a	{	12 cc. Ringer solution	b	{	7.5 cc. Ringer solution
	{	3 cc. NaCl solution		{	7.5 cc. NaCl solution
		c { 15 cc. NaCl solution			

Using phosphate buffers with pH 6–8 it was found that the KCl contracture increased with increasing pH (Fig. 3). Finally we observed that the KCl contracture is diminished by increasing the osmotic pressure and that it is more feeble in an isotonic solution than in a hypotonic one.

The smooth muscle (frog's stomach) is dependent on the chemical composition of the solution in exactly the same way as the striated muscle. There are, however, a few salts whose influence could not be tested because they themselves cause a contracture of the muscle: for this reason, Ba among others could not be used. But it could be shown that alkali chlorides favor the K contracture according to the series $\text{Li} < \text{Na} < \text{Cs} < \text{Rb}$, and that Ca and Sr are effective in the opposite sense. Still more effective are the heavy metal ions Cu^{++} , Co^{++} , Cd^{++} , and Fe^{+++} . The concentrations of these ions needed to diminish or prevent the KCl contracture are exceedingly low, as can be seen from Table I (p. 119). The effects are reversible. The diminution of the KCl effect is shown not

only by a lower contracture but also by a lengthening of the latent period. The differences are more pronounced in smooth muscle than in striated muscle because of the slower reaction of the former.

The results of experiments on the influence of osmotic pressure and of $[H^+]$ upon the KCl contracture of a strip of frog's stomach agree also with the experiments, described above for the contracture of striated muscle: with increasing osmotic pressure and with increasing $[H^+]$ the contracture is diminished (6).



FIG. 3.—Sartorius muscle. At +, 1 cc. KCl solution is added.

$$\begin{array}{ccc}
 a \left\{ \begin{array}{l} 10 \text{ cc. Ringer solution} \\ 4 \text{ cc. NaCl solution} \\ 1 \text{ cc. } \frac{m}{10} \text{ Na}_2\text{HPO}_4 \end{array} \right. &
 b \left\{ \begin{array}{l} 10 \text{ cc. Ringer solution} \\ 4 \text{ cc. NaCl solution} \\ 1 \text{ cc. } \frac{m}{10} \text{ NaH}_2\text{PO}_4 \end{array} \right. &
 c \left\{ \begin{array}{l} 10 \text{ cc. Ringer solution} \\ 4 \text{ cc. NaCl solution} \\ 1 \text{ cc. } \frac{m}{10} \text{ Na}_2\text{HPO}_4 \end{array} \right.
 \end{array}$$

If the explanation of the KCl experiments given above is correct, namely, that the change in the physico-chemical composition of the medium leads to a different susceptibility of KCl because of the changed permeability of the muscle, then it is to be expected that the same rules will hold not only for cations but also for anions. Among the sodium salts NaSCN causes the strongest contracture, and therefore we chose this salt to test the effects of different compositions of the liquid in which the muscle was immersed. Essentially the same rules were found to hold. These experiments with NaSCN can be demonstrated in the form of a physiological paradox. In general, the direct sensitivity to NaSCN is rather low. One often finds that if 1/7 or 1/6, sometimes even much more, of a Ringer solution is replaced by isotonic NaSCN solution, this concentration of NaSCN fails to produce a contracture. But if this is now

TABLE I
THE EFFECT OF BI- AND TRIVALENT IONS ON THE KCL CONTRACTURE
OF THE FROG'S STOMACH

No.	Solution	KCl Contracture		Preparation	Remarks
		Latent Period sec.	Height of Contracture in mm.		
1	10,0 Ringer + 4,0 NaCl isotonic....	22	46	Fundus	2,5 cc. KCl are regu- larly added to cause the KCl con- tracture
2	The same + 0,04 1/5 isotonic CdCl ₂ .	72	25	Fundus	
3	= 1	22	55	Pars pylorica	
4	= 2	60	29	Pars pylorica	
5	10,0 Ringer + 4,0 NaCl isotonic....	43	30	Pars pylorica	3,0 ccm. KCl are added
6	The same + 0,12 1/5 isotonic CdCl ₂ .	82	20	Pars pylorica	
7	10,0 Ringer + 4,0 NaCl isotonic....	17	23	Fundus	
8	The same + 0,05 isotonic FeCl ₃	∞	0	Fundus	
9	= 7	17	26	Fundus	2,5 cc. KCl are added
10	= 7	8	43	Pars pylorica	
11	The same + 0,04 isotonic FeCl ₃	21	17	Pars pylorica	
12	10,0 Ringer + 4,0 NaCl isotonic....	9	43	Fundus	
13	The same + 0,05 CoCl ₂ isotonic....	13	38	Fundus	2,5 cc. KCl are added
14	= 12 + 0,15 CoCl ₂ isotonic.....	26	16	Fundus	
15	= 12	9	41	Fundus	
16	10,0 Ringer + 4,0 NaCl isotonic....	11	23	Pars pylorica	
17	The same + 0,06 CuCl ₂ isotonic....	30	5	Pars pylorica	
18	= 16	11	18	Pars pylorica	

* The magnification was 7,5 fold.

replaced by Ringer solution a typical contracture takes place. The proof that we have here to do with a NaSCN contracture is furnished by experiments with LiSCN, NH₄SCN, and Ca(SCN)₂, which show the same results, and further by exact determination of the isometric tension of the contracture. It was found, for instance, that in contrast to the K contracture the tension of the direct NaSCN contracture is very low (about 1.5 per cent of the tension after faradization of the muscle); almost the same low values (on the average 3.9 per cent of the tetanic tension) were observed in the paradoxical NaSCN contracture.

The phenomenon that the same stimulation takes place after diminution of the concentration of a reagent as after increase has often been observed, for instance, recently by Jendrassik (8).

That under definite conditions the NaSCN contracture is observed only in the paradoxical form can be explained by the assumption that the

NaSCN contracture takes place if the velocity with which the substance enters or leaves the cell surpasses a definite amount.

Because of the rather low permeability of the striated muscle to sodium salts no contracture is developed unless a high concentration of NaSCN is used. But NaSCN even in concentrations below the threshold for direct contracture gradually increases the permeability of the surface layer of the cell and allows more and more NaSCN to enter the cell. If now the liquid is exchanged for Ringer solution, the salt leaves the cell with such high speed that we notice a contracture.

Two groups of experimental observations favor this opinion: First, it could be shown that the paradoxical phenomenon increases with lengthening of the time during which the muscle is in contact with NaSCN. Further, if we add to the Ringer solution containing NaSCN salts like CaCl_2 which diminish the permeability, the paradoxical phenomenon decreases.



FIG. 4.—Paradoxical SCN contracture of sartorius muscle (*R. esculenta*).

Lower tracing: At the first +, Ringer solution is exchanged for 9 cc. isotonic NaCl , + 9 cc. isotonic NaSCN; at the second +, the previous solution is exchanged for 18 cc. Ringer solution.

Upper tracing: At the first +, the same is done in the experiment reproduced in the lower curve. At the second +, the solution is exchanged for 12 cc. Ringer, + 6 cc. NaCl .

Second, it could be shown that the strength of the paradoxical contracture can be influenced to a large extent merely by changing the composition of the solution which was substituted for the Ringer solution containing NaSCN. If this solution increases permeability, the paradoxical contracture increases; if, on the other hand, it decreases permeability, the paradoxical phenomenon is diminished or completely suppressed.

Figure 4 shows that the paradoxical contracture is less in Ringer

solution than in a mixture of Ringer and isotonic NaCl solution, although in both cases the muscle was previously immersed in the same Ringer solution containing NaSCN. By adding Ca or Sr or Mg the paradoxical contracture can be suppressed completely, as shown in Figure 5. But all

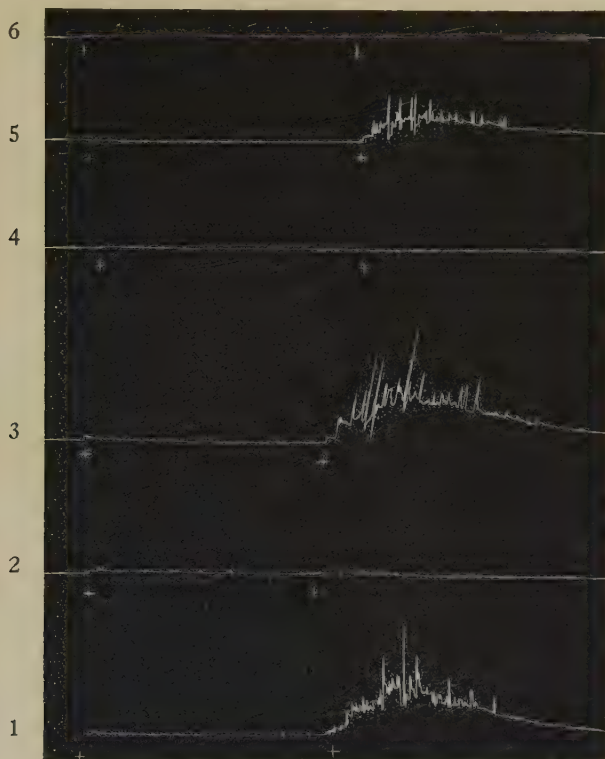


FIG. 5.—The influence of the alkali earths upon the paradoxical SCN contracture. At the first +, Ringer solution is regularly exchanged for 7.5 cc. NaCl, + 7.5 cc. NaSCN; at the second +, this solution is exchanged for 15 cc. Ringer solution in the first, third, and fifth experiment (read from below to above). But it is exchanged for the following solutions in experiment:

2 { 13 cc. Ringer solution 2 cc. CaCl_2 solution	4 { 13 cc. Ringer solution 2 cc. SrCl_2 solution	6 { 13 cc. Ringer solution 2 cc. MgCl_2 solution
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these effects are reversible, as can also be seen by Figure 5. Further experiments showed that the cations of heavy metals are still more effective than the alkali-earth cations. If the concentration of the second solution is changed, one sees that the paradoxical contracture decreases with increasing osmotic pressure. Experiments with variations of the pH lead to the conclusion that the paradoxical phenomenon increases with increasing pH. If we compare the effect of the physico-chemical compo-

sition of the solution on the strength of the paradoxical phenomenon with the influence of the same factors on the KCl contracture of the striated and smooth muscle, we recognize a close parallelism. The experiments suggest identical explanations in the two cases. If we had to do with an antagonism of ions which was not due to a change in the permeability, we would probably find that the antagonism would follow different rules in the two cases. The identical effects in these experiments can easily be understood on the assumption that the permeability of the cell determines the susceptibility of the muscle to KCl and to NaSCN in the same way.

In favor of this opinion further experiments can be mentioned in which the muscle was stimulated by single-break shocks at intervals of about 1.5 seconds. We know from other experimental work, for instance, that of Weiss (13), that stimulation of the muscle increases its permeability. If the explanation given for the paradoxical phenomenon were true, it seemed probable that the paradoxical contracture would be strengthened in the stimulated muscle in comparison with the unstimulated muscle, other conditions of the experiment being identical. The experiments showed the correctness of this idea. It was found that if the experiment was performed under conditions such that the paradoxical contracture was rather feeble, electrical stimulation increased the paradoxical reaction. (See Fig. 6.) On the other hand, if we used a concentration of NaSCN which was ineffective for the unstimulated muscle we could obtain the paradoxical phenomenon by electrical stimulation.

Because there is a close connection between the stimulation and the fatigue of the muscle, these experiments form the bridge to another group of experiments in which we studied the relation between the fatiguability of the muscle and the physico-chemical composition of the liquid in which the muscle is immersed. Embden and Adler (2) showed that the permeability of the muscle increases with increasing fatigue of the muscle by long-continued stimulation. The great importance of this change of permeability is evident if we remember the gradual transition between fatigue and death. Both are characterized by a high degree of permeability, but the difference is that in death the increase in permeability is no longer reversible. These experiments made it seem probable that a diminution of the increase of permeability during stimulation must also to a certain extent prevent or diminish the fatiguability of the muscle. On the other hand, it is clear that too great a decrease in permeability of the cell would not be favorable, because the irritability of the cell is regularly connected with the capacity of the cell to undergo increase in permeability when stimulated. These reflections were the theoretical basis of experiments directed toward finding out if it is possible to diminish the fatiguability of the muscle by increasing the CaCl_2 content of the Ringer solution bathing the muscle. The excised sartorius muscle of the frog was stimulated

with maximal or almost maximal discharges of a condenser. The frequency of stimulation was forty or more per minute, so that a decrease in the height of contraction occurred soon. Preliminary experiments (one of them reproduced in Fig. 7) proved that the two sartorii of the same frog showed under the same conditions an identical course of fatigue. Now the effects of Ringer solutions of different CaCl_2 content were studied.

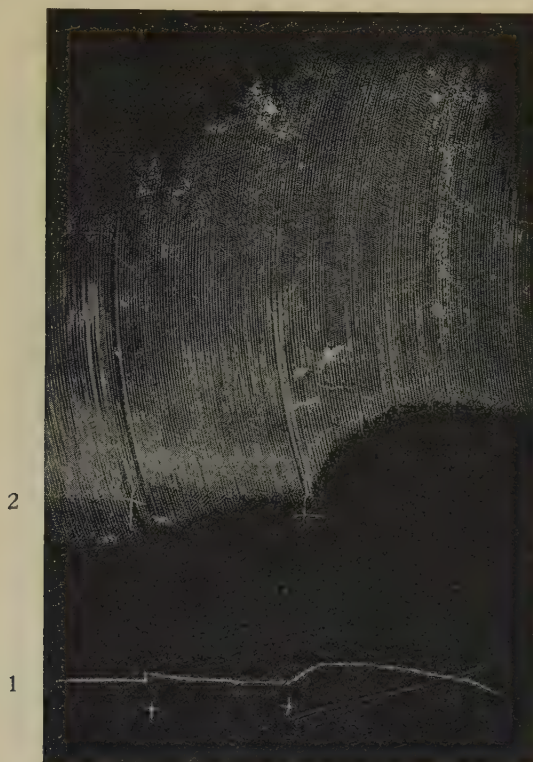


FIG. 6.—Lower tracing (without electrical stimulation): At the first +, Ringer exchanged for 12 cc. Ringer + 5 cc. NaSCN. At the second +, this solution was exchanged for 12 cc. Ringer solution + 5 cc. NaCl.

Upper tracing: The solutions are the same as in the lower tracing; but the muscle is stimulated by the discharge of a condenser 40 times per minute.

In accordance with the suggestion mentioned above it was found that in fact the fatiguability of the muscle was decreased when the muscle was suspended in a solution with increased calcium ion concentration. From Figure 8 (p. 125) it can easily be seen that in spite of the identical heights of contraction at the beginning of the experiments, the work of the control muscle slowed down much faster than that of the muscle immersed in a

Ringer solution with a higher Ca concentration. A series of experiments with different Ca concentrations was performed, with the result that increasing the Ca content to six to twelve times its original value regularly diminished the fatiguability of the muscle. This is illustrated by Table II, where the fatiguability of the sartorius muscle of the frog in solutions with different Ca content is shown. The muscles were stimulated forty times per minute and the height of contraction was determined at intervals of two minutes. These figures are given in the table, in which three experiments were selected from a greater number. The salt solutions were isotonic.

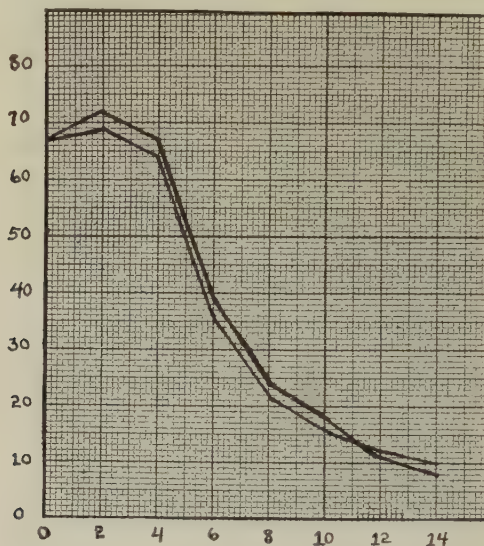


FIG. 7.—Each tracing represents the course of the change in the height of contraction of the stimulated sartorius muscle. Both muscles are from the same frog (*R. esculenta*). Stimulation identical with that of Fig. 6. Composition of the liquid in which the muscle is immersed: 19.5 cc. NaCl, 0.5 cc. CaCl_2 .

Abscissa: time in minutes; ordinate: height of contraction (in arbitrary units).

It is important to mention that in control experiments in which the muscle was not stimulated, its irritability was the same in all of the solutions applied during periods of time equal to those used in the experiments mentioned above. From this experiment the important conclusion can be drawn that *fatiguability is independent of irritability*. Further experiments supported this conclusion, for when experiments on the irritability of unstimulated muscles were carried on for relatively long periods the irritability decreased faster in the Ringer solution with a higher Ca content than in the ordinary Ringer solution. These facts are also of interest for the theory of ionic antagonism. We are not entitled to speak of an optimal balanced solution for a definite cell like the muscle cell. For it

must be specified under what conditions the muscle cell is studied. The frequently stimulated muscle cell has a quite different optimal solution from the resting one. The laws of the antagonism of ions are different in the two cases. According to further experimental observations there is a close relation between the Ca content of the solution and the Na content. The higher the Na content was, the higher the Ca content of the solution had to be in order to bring about an equal reduction in the fatiguability of the muscle. The importance of these experiments for the theory of ionic antagonism will be discussed elsewhere.

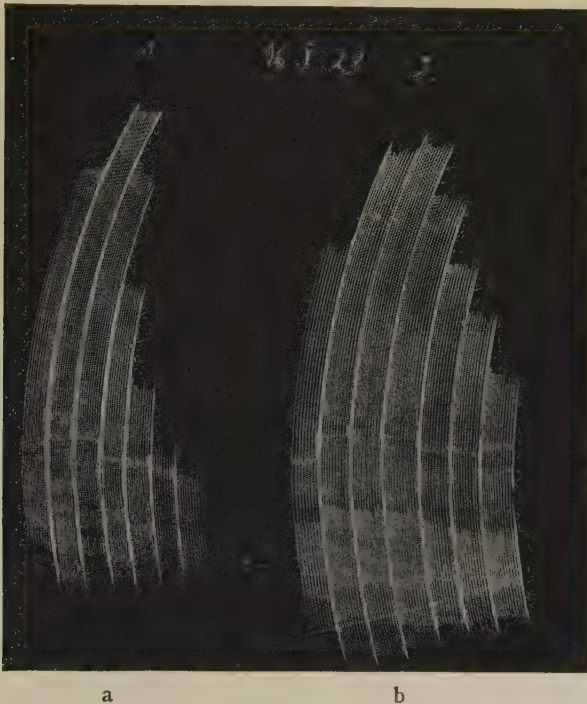


FIG. 8.—Arrangement of the experiment identical with that of Fig. 7. In the tracings the muscle was immersed in:

<i>a</i>	$\left\{ \begin{array}{l} 19.6 \text{ cc. NaCl} \\ 0.18 \text{ cc. KCl} \\ 0.20 \text{ cc. CaCl}_2 \end{array} \right.$	<i>b</i>	$\left\{ \begin{array}{l} 18.2 \text{ cc. NaCl} \\ 0.18 \text{ cc. KCl} \\ 1.6 \text{ cc. CaCl}_2 \end{array} \right.$
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In the first group of experiments described above it was shown that the ability of the striated muscle to go into KCl or NaSCN contracture is influenced in the same way by the ionic composition of the surrounding solution as that of the smooth muscle. This fact could be explained easily by assuming that in these solutions the permeability of the cell is altered.

TABLE II

Solution		Successive Responses in Experiment Number		
No.	Composition	1	2	3
I	NaCl 19,60 cc....	49, 59, 52, 35, 22, 13, 11	50, 45, 38, 25, 20, 15, 13	58, 64, 39, 8, 3, 3, 1, 5
	KCl 0,18 cc.....			
	CaCl ₂ 0,20.....			
II	NaCl 18,60.....		50, 53, 52, 44, 34, 26, 22	
	KCl 0,18.....			
	CaCl ₂ 1,20.....			
III	NaCl 18,20.....	43, 56, 58, 52, 42, 35, 25		
	KCl 0,18.....			
	CaCl ₂ 1,60.....			
IV	NaCl 17,40.....			
	KCl 0,18.....			51, 63, 60, 45, 35, 20, 15
	CaCl ₂ 2,40.....			

The susceptibility of the cell to these substances which cause contracture seems to be dependent upon the permeability of the cell. The experiments make it probable that the rules found in numerous investigations on plant cells and single animal cells hold also for the more complicated tissues of vertebrates. The latter also may be considered as rather simple material for the demonstration of the laws of permeability and their importance for function.

The second group of experiments shows that the permeability governs the reactivity of the muscle not only to substances causing contracture but also to electrical stimulations which lead to normal muscular contraction. Further experiments will be needed to prove the correctness of the thesis that the fatiguability of the muscle depends upon the permeability of the surface layers of the muscle cell.

The last series of experiments concerns the effects of internal secretions and the rôle of the autonomic nervous system in reference to permeability. A recent survey of the literature on this question (7, 5) indicated that observations made on organisms were not in agreement. Therefore an attempt was made to study the effects of internal secretions and of the autonomic poisons on the permeability of muscle under as simple conditions as possible. The effects of these substances are in the main identical with those of stimulation of the parasympathetic and sympathetic nerves. The muscle membrane made from the abdominal muscles of *Rana temporaria* according to Winterstein (16) and a sac formed from the skin of the frog according to Wertheimer (14) were used. Ringer solution containing sugar was put on one side of the membrane, on the other was

a balanced salt solution. The amount of sugar entering the membrane during different periods of time was determined by the method of Folin and Wu. Symmetrical membranes from the same frog were regularly used. In preliminary experiments it was found that the permeability of symmetrical membranes from the same frog was under identical conditions about equal. The differences did not surpass 4 per cent in the muscle experiments nor 9 per cent in those on the skin. The average was 2 per cent in the former and 3 per cent in the latter group, so it was certain that differences greater than 10 per cent caused by the application of the internal secretions or autonomic poisons must be due to a change in the permeability of the membrane.

The principal experiments made by this method showed that the effect of adrenalin on the permeability of the muscle membrane was dependent upon its concentration. If the dilution was 1:1,000,000 the permeability to sugar was increased 21–108 per cent. This enormous increase of the permeability was not due to diminution of the irritability, since the internal secretions and the autonomic poisons were always added in such small amounts that no change in the irritability of the membrane occurred. Therefore an increase or decrease in the amount of sugar which entered must be due to a specific effect on the permeability. Adrenalin in a concentration of 1:5,000,000 caused either an increase or a decrease of permeability, while in still lower concentrations (1:15,000,000) a diminution of the permeability usually occurred. A concentration of 1:50,000,000 was without effect. Corresponding results were obtained on the skin membrane. Here also a regular increase of permeability was found with adrenalin 1:1,000,000, while in lower concentrations (1:5,000,000 and 1:25,000,000) the permeability sometimes increased and sometimes decreased. The sensitivity of the skin was still greater than that of the muscle membrane: even in a concentration of 1:50,000,000 adrenalin was observed to cause a decrease of permeability.

Thyroxin caused an increase of the sugar permeability of the muscle membrane in a dilution 1:100,000 and 1:1,000,000. An increase in the permeability of the skin membrane was found regularly in concentrations of 1:10,000 up to 1:1,000,000. In lower concentrations thyroxin was ineffective.

Finally, corresponding experiments were performed with a preparation of insulin which was free from disinfectants. It was shown that insulin in concentrations of 1/50 unit per cc. up to 1/200 unit per cc. regularly increases the permeability of the muscle membrane, and the same effect was obtained on the skin membrane in concentrations of 1/50 and 1/100 unit per cc. In still lower concentrations no effect was obtained. In these experiments also, there was no perceptible change in the irritability. The experiments showed that adrenalin, thyroxin, and insulin

influence the permeability of a surviving membrane when used in about the same concentrations as those in which they exert their characteristic effects on surviving organs under similar conditions. Because of the low concentrations of adrenalin, in which adrenalin is found in the body, it is to be expected that under physiological conditions adrenalin alone decreases the permeability of the cells, while insulin and thyroxin have just the opposite effect. The observations support the conclusions drawn by Eppinger (3), Asher and Pflüger (1), and Wiechmann (15) from experiments on warm blooded animals, on man after removal of the thyroid, and in diabetes mellitus. Of course many questions are still to be solved. It will be of interest especially in reference to the theory of permeability to find out if one has here to do with a general change in the permeability independent of the nature of the penetrating substance, or if the change in permeability affects only a few substances.

An additional group of experiments was performed with pilocarpin and atropin. It was found that pilocarpin in concentrations of 1:10,000 up to 1:50,000 increases the permeability of muscle and skin membranes and that atropin in dilutions of 1:10,000 up to 1:30,000 diminishes the permeability of both membranes to sugar. A short survey of the results of experiments with internal secretions and autonomic poisons is given in Table III.

TABLE III
CHANGES IN PERMEABILITY OF MUSCLE AND SKIN MEMBRANES DUE TO
INTERNAL SECRETIONS AND AUTONOMIC POISONS

I. Muscle Membrane			
Adrenalin	1 : 1,000,000 ++ 1 : 5,000,000 + or - 1 : 15,000,000 -	Pilocarpin	1 : 10,000 ++ 1 : 50,000 +
Thyroxin	1 : 100,000 ++ 1 : 1,000,000 ++	Atropin	1 : 10,000 -- 1 : 30,000 --
Insulin	1/50 and 1/100 unit per cc. ++ 1/200 unit per cc. +		
II. Skin Membrane			
Adrenalin	1 : 1,000,000 ++ 1 : 5,000,000 + or - 1 : 50,000,000 -	Pilocarpin	1 : 10,000 ++ 1 : 50,000 ++
Thyroxin	1 : 10,000 ++ 1 : 100,000 ++ 1 : 1,000,000 +	Atropin	1 : 10,000 -- 1 : 30,000 --
Insulin	1/50 and 1/100 unit per cc. ++		

* The + means increase, and - means decrease in permeability for sugar.

The experiments suggest that in the body also the internal secretions and the autonomic nervous system take a part in the regulation of the permeability of the cell. It is of interest to see that the antagonism between the effect of pilocarpin and atropin, on the one hand, and between pilocarpin and adrenalin, on the other hand, is confirmed in these observations on permeability.

If we review the experiments discussed in this paper we see how different are the possibilities of varying the permeability of the cell. The smallest changes in the concentrations of ions are effective in this respect, as are also stimulation of nerves including the autonomic system and the specific secretions of internal glands. In organisms all these processes may interfere with one another. An example of this was given in the fatigue experiments in different solutions. Therefore we see innumerable ways by which the permeability of the cell can be regulated and adapted to different conditions. The close dependence of the functions of a cell upon the behavior of the permeability of its cell membrane makes it clear that the organism uses different means to attain this end. For we have here to do with a fundamental physiological process.

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XI. THE ACCUMULATION OF MINERAL ELEMENTS BY PLANT CELLS

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GENERAL NATURE OF PROCESS OF ACCUMULATION

One of the apparently almost universal characteristics of vacuolated plant cells is their ability to accumulate, and to maintain in the cell sap, certain inorganic elements in concentrations far exceeding those found in the culture medium. Usually the sap is also entirely unlike the culture medium with regard to the relative proportions of the different elements present in solution. We may ask, first, whether these relations between the sap and the culture medium can be described entirely in terms of cell permeability. Obviously, at some period the living cells must be permeable, and differentially permeable, to those elements found within the cell. The permeability may be slight; in other words, the resistance to the passage of a solute across the protoplasm may be much greater than in the case of certain types of artificial cells, as indicated by experiments of sufficiently short duration. Yet, during its growth, the living cell gradually differentiates itself from its environment in a manner very unlike that of a simple artificial cell. In this particular discussion we are dealing, therefore, not merely with resistance to diffusion of solutes through a membrane, or with degrees of permeability, but also with phenomena associated in some fashion with the dynamic processes of cell growth or metabolism.

The general situation described above is fairly familiar and I need recall only a few characteristic illustrations. Some investigators now present will be especially interested in the relations of marine organisms to their environment. Many years ago our laboratory in Berkeley had occasion to make chemical studies on very carefully collected samples of some of the giant kelps of the Pacific Coast, particularly *Macrocystis pyrifera* and *Nereocystis luetkeana* (3). It became evident that these plants had accumulated potassium and sodium within their cells in proportions radically different from those existing in the surrounding medium of sea water. Iodine, also, had been accumulated in very appreciable amounts, although the concentration of this element in sea water is exceedingly small. A marked accumulation of bromine was likewise demonstrated later. In the case of all these elements, the evidence suggested that they were present in the plant for the most part in a dissolved and inorganic state.

¹ This paper refers to investigations conducted in co-operation with A. R. Davis, P. L. Hibbard, J. C. Martin, and T. C. Broyer, in the Laboratory of Plant Nutrition, University of California.

A number of years ago Osterhout (12) presented data on a marine alga of exceptional physiological interest, a species of *Valonia*. This organism produces single cells (single from a physiological point of view, even though multi-nucleate) of relatively enormous size, so that almost uncontaminated cell sap can be obtained in sufficient amounts for chemical analysis, even from a single cell. The results secured at Osterhout's suggestion gave a direct insight into the general composition of the two media, namely, cell sap and sea water, which were separated from each other essentially by a cell wall and a very thin layer of protoplasm. The *Valonia* sap was found to have an entirely different composition from that of the sea water. Especially notable was the concentration of potassium in the sap, which was about 50 times higher than in the sea water, while sodium was less concentrated in the sap than in the sea water. Recently, R. Höber and J. Höber (8) also have studied the composition of *Valonia* cell sap, at Naples, and have obtained similar results. Extensive chemical data on *Valonia* cells have been reported by S. C. Brooks (2).

The investigator of land plants comes in almost daily contact with illustrations of the accumulating power of plant cells. Plants of this type normally grow rooted in soils, in which the liquid phase is very dilute in character, and solutions similar to soil solutions may be employed successfully as artificial culture media. The sap which can be expressed from the plant tissues, whether of roots, stems, or leaves, usually contains the principal mineral elements in concentrations far higher than those found in the culture medium. The relative, as well as the absolute concentrations of the mineral elements of the sap are also very different from those of the culture solution.

In the marine organisms which have been referred to, the total concentration of electrolytes in the sap may not be very different from the total concentration in the sea water, even though certain elements, particularly potassium, may exist in far higher concentration in the sap than in the sea water. Certain other elements or radicals may have a higher concentration in the sea water than in the sap. On the other hand, it is generally found with land plants, and with fresh-water plants, that the total concentration of electrolytes, and the individual concentration of each important element, are very much higher in the sap than in the culture medium. One difficulty in establishing such relations accurately is that it is impossible to obtain from complex plant tissues sap of clearly definable origin. Six or seven years ago, our attention was turned to the fresh-water alga *Nitella* (*Nitella clavata*), which produces cells of sufficient size to be manipulated as separate units, although these cells are far smaller than *Valonia* cells. Nevertheless, for our purposes, *Nitella* cells present a distinct advantage, in that they normally grow in solutions that are not fundamentally unlike many soil solutions, so that definite comparison

with plants grown in soil becomes possible. It is also possible to subject *Nitella* cells to greater changes in their solution environment than is the case with *Valonia* cells.

The composite sap obtained by the process of breaking individually large numbers of *Nitella* cells (of 1 to 3 inches in length) was subjected to chemical analysis. Collections of cells were made at different seasons of the year. It was always found that the cell sap had a conductivity about 25 times greater than that of the pond water in which the cells grew, and that each of the principal ions was present in much higher concentration in the sap than in the culture medium (4, 7). Unlike *Valonia* cells, *Nitella* cells accumulate calcium, magnesium, sodium, and sulphate in the cell sap to a marked degree. In some collections sodium was present in greater equivalent concentration than potassium, though the concentration factor was much higher for potassium than for sodium. The various concentration factors (concentration in sap divided by concentration in culture medium), are illustrated in Table I. For the present purpose concentra-

TABLE I
COMPOSITION OF CELL SAP OF *Nitella* COLLECTED AT DIFFERENT TIMES*

Dates of Collection†	Composition of Sap (milli-equivalents)‡								
	Cl	SO ₄	H ₂ PO ₄	Sum Anions	Ca	Mg	Na	K	Sum Cations
1924									
August 9–Sept. 25	106.5	20.2	2.4	129.1	19.3	17.2	78.8	58.2	173.5
1925									
Sept. 23–Sept. 26	101.1	13.0	1.7	115.8	13.0	10.8	49.9	49.3	123.0
August 22–Sept. 12	105.5	11.9	2.0	119.4	11.7	12.1	56.2	50.8	130.8
Nov. 5–Nov. 21	102.9	17.9	2.8	123.6	11.5	16.4	40.0	59.1	127.0
April 29–May 21		21.0	3.5		18.7	21.7	85.7	43.5	169.6
Pond water, average composition, samples of August 9 and September 26, 1925.§...	1.0	0.67	0.0008		1.3	3.0	1.2	.05	

* The conductivity of the sap was approximately 25 times that of pond water. The pH of the sap was uniformly very close to 5.2; the pH of the pond water varied from 7.0+ to 9.0+, depending upon the utilization of CO₂ by the plants.

† First date, time of collecting cells from pond; second date, time when cells were taken from tank at laboratory, and sap obtained. During the interval the cells were kept in tap water very similar in composition to the pond water.

‡ Values expressed as milli-equivalents per liter of sap or of culture solution.

§ HCO₃ ion also present in pond water in relatively large concentration. NO₃ also present in water but not in sap.

tions are expressed in terms of milli-equivalents per liter, but unpublished calculations made by P. Zscheile, of this laboratory, show that much interest may be attached to a consideration of ionic strengths and ion activities. This method of expression modifies certain of the relations between different ions, and provides a more accurate idea of the thermodynamic system.

The pH value of the sap of healthy cells was found to remain nearly constant within experimental error, at pH 5.2, although the outside medium (pond water) was much more alkaline and of varying reactions (pH 7.0 to 9.0+), depending upon the photosynthetic utilization of carbon dioxide. The relative constancy of reaction in the sap is not simply a matter of buffering. The buffer effect of *Nitella* sap is not large and could not long maintain the constant acid reaction in the absence of other mechanisms.

According to certain older views, the accumulation of mineral elements by plant cells was explained on the basis of chemical combinations or adsorptions occurring within the cell, which removed the elements from solution until a condition of diffusion equilibrium was attained. The condition existing in *Nitella* cells, and in similar plant cells, admits of no such simple explanation. The conductivity of the sap, the approximate chemical equivalence of simple cations and anions, the slight amount of organic matter present in the sap, and the osmotic relations existing between the cell and its medium, all point to the conclusion that the sap is essentially a solution of electrolytes with a conductivity approximately that of a M/10 KCl solution. Attempts have been made occasionally to invoke the aid of the Donnan equilibrium in explaining the phenomenon of accumulation. Time is not available for a discussion of this point, but I believe that a detailed examination of the data will make it obvious that the Donnan equilibrium conditions are in no way fulfilled by the sap-culture medium system. This conclusion is also reached by S. C. Brooks (2) and by Osterhout (13), on the basis of experiments on *Valonia* cells.

EXPERIMENTS ON ACCUMULATION OF HALIDES BY *Nitella* CELLS, WITH REFERENCE TO LIGHT, TEMPERATURE, AND OXYGEN

While the relations existing between *Nitella* cells and their natural medium of pond water are of great interest, it is not possible to proceed far without a study of accumulations taking place under controlled conditions. We have conducted many such experiments, especially with solutions of bromides. The bromine ion was selected for the following reasons: (a) it is not normally present in the sap; (b) it is relatively non-toxic; (c) it is capable of marked accumulation; (d) satisfactory methods of analysis could be developed.

Since the processes of accumulation involve a movement of solutes

against concentration gradients, or some equivalent effect, it seemed important to make observations to determine the relation of metabolic processes to accumulation, on the assumption that expenditure of energy must be involved, directly or indirectly. The influence of light and temperature was, therefore, given attention very early in the course of the researches. It was found consistently that bromine ions could be accumulated in the cell sap from outside solutions of .005 M KBr (buffered or unbuffered), until internal concentrations were attained far in excess of the outside concentrations, but only provided the cells were subjected to illumination (5, 7). (The periods of the experiments were, for the most part, 3 to 10 days in duration.) The highest accumulations took place when the cells were exposed to continuous strong illumination, with bromine present in the solution; but definite accumulation also occurred during dark periods, immediately following periods of high illumination. Cells maintained continuously in the dark after removal from a low light condition were certainly permeable to bromine, since the latter entered the cell sap, but there was little or no accumulation; that is to say, concentrations attained in the sap were not very different from those of the outside solution. Injury to cells occurred under prolonged periods of light exclusion, especially when no provision was made to aerate the solution continuously. The effects of oxygen will be mentioned presently.

When cells were exposed to conditions of relatively intense artificial illumination for periods of 12 or 24 hours without access to bromine, alternated with equal periods of darkness with access to bromine, it was discovered, as noted above, that significant accumulations of bromine could take place during the periods of darkness. In other words, there was a residual light effect. Such accumulations were of considerably smaller magnitude than those occurring when bromine was available to the cells during the periods of illumination.

While the majority of experiments were made with bromide solutions, it is worthy of note that iodine also could accumulate in the cell sap in concentrations far exceeding those of the culture solution. Gradual injury to cells was usually found to occur following high accumulations of iodine ions, conceivably as a result of the formation of a small amount of molecular iodine. In addition to accumulations of bromine and iodine, it was possible under proper conditions to increase the chlorine concentration normally present in large cells by as much as 20 or 25 per cent. An approximately equivalent increase in potassium concentration took place concurrently. The conductivity of the sap showed an increase roughly proportional to the increase in electrolytes, as determined by chemical analysis.

With a given condition of illumination, variations in temperature pro-

duced significant alterations in the amounts of bromine accumulated over a given period of time. The temperature coefficient was of a high order, in excess of 2 for 10°C. increment. At about 5°C., it is probable that accumulation sinks to a very low or negligible value. In certain experiments we have noted a slight outward diffusion of chlorine when the temperature was lowered to 5°C. Subsequently, when the temperature was raised, the cells were able to re-absorb the chlorine lost. Above 25°C. effects of injury are likely to intervene.

The ability of the cells to accumulate bromine was greatly reduced by injurious substances, such as chloroform and cyanide, etc. It is possible that injury may have increased the general permeability, per se, at the same time that ability to accumulate ions decreased. Certainly, when injury occurs, there is a tendency for chlorine to diffuse out of the cell sap into the surrounding solution. It does not appear that loss of chlorine takes place from healthy cells except as a result of certain ionic exchanges, to be referred to presently.

At this point in the discussion, it is pertinent to mention some preliminary experiments on an entirely different type of plant tissue, conducted in Berkeley recently by Dr. F. C. Steward, of the University of Leeds (unpublished). In these experiments potato tissues were employed, cut in very thin discs of accurately controlled thickness. Elaborate provision was made by Steward for control of the gaseous environment of the tissues, a vital feature of such experiments. While the experiments are in an initial stage of development only, it is clear that the accumulation of bromine by the potato tissues was fundamentally influenced by the gaseous environment. (Accumulation of potassium was also demonstrated.) It was possible to cause a rapid accumulation of bromine in the sap expressed from the plant tissues, but this accumulation was dependent on adequate aëration. When the percentage of oxygen in the oxygen-nitrogen mixture was low, for example, 2.5 per cent, very little accumulation occurred. With increasing percentages of oxygen there was increasing accumulation, up to possibly a very high percentage of oxygen. Under the most favorable environment practically every trace of bromine could be removed from solution by the tissues.

Previous experiments with *Nitella* cells invariably showed a tendency for bromine accumulation to be decreased when air enriched by additional carbon dioxide was passed through the culture solution. Some of the higher admixtures of carbon dioxide caused evident injury. On the basis of Steward's experiments it was thought desirable to study the influence of passing oxygen through the solution surrounding the cells. Only a few results are available as yet, but one or two experiments showed that accumulation of bromine in the dark was made possible by passing a rapid stream of oxygen through the solution, although this accumulation was still

much smaller than that occurring in the illuminated cells without the use of oxygen. (The passing of oxygen maintained cells in the dark in better condition.) It is obvious, of course, that the potato tissues have a large reserve of carbohydrate susceptible of oxidation, while *Nitella* cells are more or less immediately dependent on light as a source of energy.

The rôle of illumination as influencing accumulation of electrolytes by *Nitella* cells is not now capable of elucidation. For reasons that have already been sufficiently indicated, it seems entirely inadequate to say merely that cell permeability is increased. Zycha (15) in 1928 reviewed earlier work on the effects of light on permeability and described his own experiments. He was not able to obtain definite confirmation of light effects. It is to be noted that the experiments on permeability, usually made by plasmolytic methods with the use of strong solutions, are not comparable with our experiments on accumulation from dilute solutions, taking place during relatively long intervals of time.

Concerning the part played directly or indirectly by photosynthetic reactions and the formation of energy containing compounds, no definite statement can be made, other than noting that residual effects of light were observed in the case of *Nitella*, while root cells carry out accumulation entirely in the dark. In the latter instance direct permeability effects of light are excluded. More study is required on the oxygen-carbon dioxide relations of *Nitella* cells, as related to illumination. Experiments in this direction are now under way. It is interesting, although perhaps not profitable, to speculate on the possible rôle of electric potentials set up as a result of oxidation-reduction reactions.

It is hoped that the foregoing remarks will emphasize the importance of the relation between plant metabolism, energy utilization, and ion accumulation. This general question is of utmost importance, not alone to the plant physiologist but also to the animal physiologist. Entirely analogous problems must be faced by the latter. Recently Netter (11) has considered the equilibrium of electrolytes in various animal tissues and has reached the conclusion that metabolic activities must be taken into account. He emphasizes the production of hydrogen and ammonium ions, which he believes can be exchanged for potassium ions. Other illustrations of problems in animal physiology are cited by S. C. Brooks (2).

INTERRELATIONS OF IONS IN PROCESSES OF ACCUMULATION OF *Nitella* AND OTHER PLANT CELLS

There remain to be considered the relations existing between the composition of the culture solution and accumulation, assuming that other environmental factors, namely, light, temperature, and gaseous environment, are favorable. We can merely outline a few of the observations which have

been made (6, 7). Early in the investigations, we were impressed by the interrelations existing between certain anions, and especially between bromine and chlorine. When *Nitella* cells in a thoroughly healthy condition were placed in certain non-toxic solutions, or sometimes even in distilled water, it was found that the chlorine already present in the cells in large concentration did not pass out of the cell into the outer medium. If, by any means, the cells were injured, then such outward diffusion did take place readily. When bromine was present in the culture solution, even in the absence of observable toxic conditions, there was a marked loss of chlorine from the cell, accompanying accumulation of bromine in the cell sap. In effect, an exchange of bromide and chloride ions occurred. (R. Höber and J. Höber, 8, have reported recently some experiments indicating that bromine-chlorine exchange can be made to take place in *Valonia* cells.) When chloride ions and bromide ions were both present at the outset in the culture solution, the accumulation of bromine was strongly retarded by the chlorine present. These effects occurred even in very dilute solutions, of the order of .001 molal. In one experiment, when .03 molal KCl was present in the culture solution containing .001 molal KBr, the accumulation of bromine in the sap at the end of a five-day period was less than one-tenth of that occurring from a solution containing only KBr.

Perhaps it will be thought that the accumulation of bromine is always an exchange of ions in chemically equivalent amounts. Our experiments show that this is not a correct picture. In addition to the bromine which is accumulated by exchange, relatively large amounts may be accumulated by a process which involves the simultaneous accumulation of a cation. If calcium is the only cation available, the indications are that most of the bromine accumulation is of an exchange nature, since calcium, unlike potassium, cannot be increased appreciably in its concentration in the cell sap, at least over any limited period of time, such as ten days. Anions, such as sulphate, nitrate, or phosphate, which are accumulated with difficulty by the large, more or less mature cells, such as were used in these experiments, have little or no influence on the accumulation of bromine. It appears, in other words, that one anion cannot influence the accumulation of another, unless it itself has the potentiality of relatively rapid accumulation in the sap. These facts mean, presumably, that the interrelations between anions now under consideration do not become effective at the outer surface of the cell.

The accumulation of an anion, as represented by bromine, may be greatly influenced by the character of the cation. The suggestion yielded by the data now available is that the maximum rate of bromine accumulation occurs only when an adequate concentration of a rapidly accumulating cation is also present in the solution. The amount of bromine accumulation taking place over a given period of time was found to be much smaller with

solutions of CaBr_2 , LiBr , SrBr_2 , and MgBr_2 than with solutions of KBr and RbBr . NaBr occupied an intermediate position.

The relation of cation to cation is not easily studied with *Nitella* cells. It is very difficult to induce additional accumulation of sodium, calcium, or magnesium, and in the case of sodium, the analytical methods offer an obstacle. Probably some exchange of sodium and potassium ions can take place, but not nearly so easily as the exchange of bromine for chlorine. In the dilute solutions of KBr employed, calcium, unless present in relatively high concentration, seems to have little influence on the accumulation of bromine or potassium.

The interrelations of cations may be readily investigated by the use of certain crop plants, such as barley. The results of numerous experiments by different investigators show that a reciprocal relation often exists between calcium, magnesium, and potassium. This is especially evidenced by comparisons of the composition of plants grown in solutions of different potassium concentrations. When the potassium concentrations are low, some plants accumulate much more calcium and magnesium than from a solution of higher potassium concentration, both solutions having the same calcium and magnesium content. These relationships will be apparent in the composition of the sap expressed from the plant tissues, as well as in the composition of the plant as a whole.

Concentration effects in accumulation were studied with *Nitella*, using bromide solutions, and compared with similar effects reported for other ions and for different plant tissues. If concentrations of an ion present in the culture medium are plotted against concentrations present in the cell sap, after the lapse of a suitable interval of time, a logarithmic type of curve is generally given. The results can be fitted to one of the general adsorption equations, but it is not apparent that this procedure leads to any important gain in our understanding of the basic phenomena involved, and it may lead to erroneous conceptions. A logarithmic type of curve was also obtained in our experiments when intervals of time were plotted against increases of Br concentration in the cell sap. A long period, such as several weeks, may be required to reach the point at which the approximately maximum concentration of bromine in the cell sap is reached. This depends upon light, temperature, and condition and age of cells.

SELECTIVE ACCUMULATION AND ANTAGONISM

The "selective" accumulation of mineral elements by plants offers for study some interesting scientific as well as practical problems. It is well to note that the selective action by plants is not one which necessarily excludes from the plant elements of a useless or injurious character. For example, a barley plant is capable of accumulating a very high concentration of chlorine when the latter is present in the medium in sufficient amount,

although chlorine is not essential to the growth of this plant, at least in more than minute quantities. Still it is true that marked selectivity exists. Especially interesting and important is the ability of many plants to remove nitrate from solution at a rapid rate. In certain experiments barley plants removed in a given period from calcium nitrate solutions several times as many equivalents of NO_3^- as of Ca. Equilibrium was maintained by the excretion or formation of bicarbonate ions. It is possible to regard this process as an exchange of HCO_3^- ions for NO_3^- ions. A similar exchange of HCO_3^- for Cl^- may be demonstrated, but to a much less pronounced extent. These relations do not apply to all types of plants. Certain plants may absorb Ca as rapidly as or even more rapidly than NO_3^- .

Potassium is removed from solution much more rapidly than calcium by many plants, but some plants remove calcium with equal facility and much of the calcium may sometimes be stored in the plant in dissolved form. To give a specific example, *Melilotus alba* plants were grown in a sand culture with the use of a nutrient solution. After the plants had made considerable development, sap was expressed from the leaves, and analyzed. There was found to be present in milligram equivalents per liter 240 of Ca, 110 of Mg, and 76 of K. Barley plants grown under the same cultural conditions would yield sap containing K in marked preponderance over Ca and Mg.

If we survey all of the data, it is difficult to find any general physical chemical properties of the ions involved which explain consistently the relative rates of accumulation of different ions. The cations often fall in the order of their ionic mobilities in free solution, but not always. The relations of anions, complicated by metabolic changes, are very confusing. Of considerable interest in this general connection are the observations of Michaelis (9), that ions, in passing through certain types of membranes, have their differences in mobility greatly exaggerated.

I desire to call your attention once more to the fact that the phenomena of which I have been speaking throughout occur in relatively very dilute solutions, the "nutrient" type of solution. It appears that we are dealing, not with marked changes in the general permeability of the cell, but rather with specific relations between ions, or between ions and the protoplasm. Many features of these relations are very different from those described in the well-known older reports of experiments on antagonism, which have been particularly concerned with cations and with strong solutions.

We may recall that it is possible to grow plants of many types equally well in a variety of culture solutions and to bring about marked changes in the composition of the expressed sap, or of the tissues as a whole, as has already been indicated in referring to the interrelations between K, Ca, and Mg. Yet it is reasonable to assume that normal permeability of plant tissues is present under all of the various culture conditions, which result

in the growth of normal, healthy plants. In experiments dealing with antagonism of ions, it is generally sought to demonstrate that certain types of strong solutions produce marked alterations in normal cell permeability, often accompanied by injury. As another illustration of the phenomena involved, an experiment may be cited in which masses of small, healthy *Nitella* cells were immersed in KNO_3 solutions of varying concentrations, with and without the addition of $\text{Ca}(\text{NO}_3)_2$. In the lower concentrations, up to .03 M, no injury was caused by the single salt solutions, and no chlorine was found to diffuse out of the cells. In the higher concentrations, however, especially at about .06 M KNO_3 , injury occurred, accompanied by loss of chlorine from the cell sap. Such injury and loss of chlorine was prevented by the addition of a small amount of $\text{Ca}(\text{NO}_3)_2$ to the solutions.

EFFECTS OF HYDROGEN ION CONCENTRATION

Considering now some of the effects of hydrogen ion concentration on accumulation, it is true that with various crop plants it is possible to bring about striking accumulation (in roots, leaves, or stems) of cations and anions, especially of potassium and nitrate, at a variety of hydrogen ion concentrations in the culture media. It is often possible to obtain good growth and rapid accumulation at a reaction as acid as pH 5. As far as can be judged by available methods, the accumulating plant cells of many plants would contain sap of distinctly lower hydrogen ion concentration than that represented by pH 5. The pH value might often be as high as 6. It is possible, however, to decrease the hydrogen ion concentration of the culture medium until it is more alkaline than the sap, and still have marked accumulation; that is to say, this process may continue whether the solution is more or less acid than the sap.

The normal condition with *Nitella* or *Valonia* cells is for the sap to maintain a higher hydrogen ion concentration than that of the medium in which the cells live. It is not easy to study hydrogen ion effects with *Nitella* cells, because of the difficulty of maintaining a desired hydrogen ion concentration without too great injury to the cells. Nevertheless, we have been able to vary the hydrogen ion concentration of special media and observe the accumulation of potassium and bromine in the cell sap over fairly extended periods of time. It is evident that accumulation may take place over a wide range of hydrogen ion concentration—from approximately pH 5 to pH 8, or higher. The optimum point, if one exists, is too difficult to decide with present data. Some of the indications are that values around pH 6 are especially favorable. As M. M. Brooks (1) points out, a question of rate is involved, and we have not thus far been able to obtain data on which rates of accumulation, as affected by hydrogen ion concentration, could be established.

It may be of interest to quote some data recently obtained on accumu-

lation from a decidedly acid solution. The *Nitella* cells were immersed in a phosphate buffer solution of practically the same pH as that of the cell sap (pH 5.2) and exposed to strong illumination. At the beginning of each 24-hour period, the solution was adjusted to a pH of 4.9–5.1. By the end of the 24-hour period the value had increased to pH 5.3, but for the greater portion of the time the value did not exceed pH 5.2. At the end of 147 hours the experiment was concluded and the sap obtained from the larger cells was found to contain 64 milligram equivalents of bromine, and to have increased its potassium concentration by 28 milligram equivalents, about 30 per cent above the original value. Consistently with previous experiments, nearly 28 milligram equivalents of bromine must have accumulated simultaneously with an equivalent amount of potassium, the rest of the bromine accumulated being accounted for by chlorine-bromine exchange. If we can judge from previous experiments, only a small part of the potassium accumulation would have resulted from sodium, calcium, or magnesium exchange. Since there was no excess of hydrogen ion concentration within the cell sap, exchange of hydrogen ion for potassium ion was not involved, apparently. The solutions used in this experiment were slightly too acid to maintain the mass of cells in good condition throughout the period of the experiment, although the large cells from which sap was obtained were turgid and normal in appearance. Some of the various observations on hydrogen-ion relations may have a bearing on the theory of accumulation developed by S. C. Brooks, and may suggest the need for development of this theory to cover certain artificial conditions for *Nitella* cells, as well as normal conditions for other types of plant cells.

GENERAL CONSIDERATIONS

The discussion thus far has been concerned with typical electrolytes in dilute solution. The suggestion naturally occurred to attempt to determine whether accumulation of non-electrolytes, or extremely weak electrolytes, could be brought about. With several common sugars, we could obtain no evidence that they penetrated the cell, but urea seemed to penetrate readily. At first it appeared that urea was accumulated in the cell sap, but further experimentation gave evidence that urea as such was probably not accumulated, but possibly some other compound easily capable of giving up ammonia from an alkaline solution. It is still undetermined to what extent ammonium salts are accumulated under these conditions.

Many of the general facts concerning the accumulation of electrolytes by plant cells are reasonably clear, but the mechanism of accumulation involves many questions which are not subject to direct and unambiguous experimentation. Osterhout (13) has suggested that primarily only undissociated molecules are capable of penetration into living cells, with subsequent dissociation taking place in the interior of the cells. The data which

I have surveyed in this paper cannot constitute any direct evidence of the nature of the mechanism involved in the accumulation of mineral elements, but it is not clear how we can explain on a non-ionic basis certain phenomena accompanying accumulation, such as the exchange of bromine and chlorine by *Nitella* cells, the influence of cation on anion intake, and the influence of one cation on the intake of another. All of these effects may occur in very dilute solutions, in which dissociation is considered to be complete, or nearly so. Other evidence of ionic intake by plants has been reviewed recently by Pantanelli (14). S. C. Brooks (2) has developed a cell mechanism which is based essentially on an ionic hypothesis, involving ionic exchanges. He indicates certain difficulties in explaining the relative rates of penetration of potassium and sodium, if ionic penetration is excluded. Northrop (10) has just published a description of experiments with an artificial cell, practically impermeable to ions, but the latter are produced internally by certain types of chemical reactions. It is recognized that these reactions do not occur in living cells, but it is suggested that some sort of analogous process may take place. It is not now evident how the systems described by Northrop can apply to the phenomena under discussion in this paper. None of the proposed mechanisms seem to dispense with the necessity of taking into account the metabolic changes occurring in the cell. The dynamic system governing the accumulation of solutes by plant cells is not at all as simple as many texts would lead us to believe, whatever the mechanism may be. However, I believe that Dr. Brooks is to consider the question of cell mechanisms, and I leave the discussion to him, without reluctance.

SUMMARY

1. The general nature of the process of accumulation of mineral elements by various types of plant cells is briefly reviewed.

2. Previous work and new evidence emphasize the importance of metabolic processes as indispensable to the accumulation of mineral elements by plant cells. Reference is made to light, temperature, and oxygen effects, especially as shown by experiments on *Nitella* cells.

3. The effects of one ion on the accumulation of another are shown to occur in dilute solutions, and to include interrelations between ions of like charge and of unlike charge. Comparison is made with experiments on antagonism of ions.

4. The effect of hydrogen ion concentration on the accumulation of cations and anions by various types of plants is considered. Accumulation of cations and anions may take place over a wide range of hydrogen ion concentration in the culture media of many types of plants. Some experiments suggest that a gradient of hydrogen ion concentration between the cell sap and culture medium is not of necessity required.

5. It is recognized that the results reported are not capable of elucidating the actual mechanism of protoplasmic activity which brings about accumulation of mineral elements in the cell, but it is believed that at present preference should be given to some type of ionic theory of penetration.

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XII. THE YELLOW PIGMENTS OF GREEN LEAVES: THEIR CHEMICAL CONSTITUTION AND POSSIBLE FUNCTION IN PHOTOSYNTHESIS

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The discovery of Ingen-Housz (22), in 1799, that the green portions of the plant, the leaves and petioles, can assimilate carbon dioxide and liberate oxygen in the presence of light, stimulated investigators in plant physiology and chemistry to a closer analysis of the mechanism of the process. Grotthus (1) enunciated his law of photochemical action (1818), that only light which is absorbed is chemically active. This simplified the matter for the chemist because he now knew the substance for which he was to search must be colored. In consequence all pigments were for the time regarded as possessing the potentialities for the accomplishment of this photolysis.

Further investigations showed, however, that photosynthetic activity could be correlated best with the pigments which absorb red light; hence the green pigments were viewed as the active photosensitizers in this assimilatory process. Work on these green pigments, called chlorophyll *a* and chlorophyll *b*, was begun early in the history of photosynthesis and has continued to the present, culminating in the brilliant physiological (27) and chemical investigations (26) of Willstaetter and his co-workers, and in the recent synthetic researches of Hans Fischer and his colleagues (7).

While it was found that chlorophyll probably plays the most important rôle of all the pigments in this process, it was also found that it stands in a constant relationship to carotene and xanthophyll in the living leaf (27*b*). This observation stimulated anew the interest in the physiological function and chemical constitution of these yellow substances. Further investigations showed that these yellow pigments were always present in plant materials. This universal distribution indicated that they must be of some use to the plant, and so any number of theories have arisen as to their function.

These theories, which may be classified into four classes, propose that they act either as respiratory pigments, as reserve food material, as protective pigments, or as photosynthetic pigments.

1. Because of their great affinity for oxygen, Arnaud (19*a*) assumed that they played the same rôle in plants that hemoglobin plays in animals. Kohl also ascribed to carotene and xanthophyll a respiratory function (14*b*).

The fact that carotene, $C_{40}H_{56}$, stands in a constant relationship to xanthophyll, $C_{40}H_{56}O_2$, seemed to substantiate this view. On the other

hand, the fact that neither pigment has been converted into the other by any simple oxidation-reduction reaction indicates that the same relationship does not exist between these pigments that exists between hemoglobin and oxyhemoglobin. Furthermore, oxidation by the air causes deep-seated changes which are not reversible. The respiratory function seems then to be untenable.

2. The second function which has been ascribed to these pigments is that of a reserve food material. Zopf proposed this theory because he found them in greatest concentrations in the spores of plants (14a). Such a function also seems improbable because these pigments are present in relatively small amounts as compared with other food materials.

The recent discovery of the vitamine A activity of carotene (4) makes its function as an accessory foodstuff in the plant as well as in the animal seem possible although no experimental proof exists that organic substances can play such a rôle in plant life.

3. Since these pigments absorb light in the blue and violet regions of the spectrum, Kohl (19b) has proposed that they may protect the cell enzymes from photochemical inactivation and destruction. Willstaetter (27a) also says, "It is possible that they belong to an arrangement which protects the chlorophyll from photo-oxidation."

Too little is known concerning this function to evaluate its worth. The small concentration of these pigments, relative to other colorants which absorb in the same region, makes it seem improbable that such can be their function.

4. The last and to us the most important hypothesis concerning their action in the plant is that they play some rôle in the photosynthetic process. Two theories have been advanced, one that they act indirectly, i.e., through secondary reactions; the other that they act directly as a photosensitizer for carbon dioxide assimilation.

Engelmann (19a) supposed that carotene acted indirectly by changing light into heat energy, thus increasing the temperature and consequently the reaction rate.

Ewart (19c) proposed that through photo-oxidation carotene and xanthophyll passed directly into formaldehyde and sugars and in this way became a part of the photosynthetic mechanism.

Willstaetter (27a) summarized his ideas in this statement: "If the carotenoids have an especial function in the assimilation process it must be indirect and cannot be conditioned through their light absorption."

A direct photosynthetic action has been proposed by both Kohl and Warburg.

A quotation from Kohl (14d) gives his opinion on this matter: "It is possible to determine whether carotene is able to reduce carbon dioxide alone by the use of etiolated leaves. I have investigated therefore first by

the microscopic bubble-number method and second by the bacterial method, the cells and tissue [pieces] of etiolated leaves from various plants, . . . as to their ability to liberate oxygen in the light and I have obtained positive results exclusively."

In the most recent and most reliable work in photosynthesis Warburg (24) and his collaborators make it seem probable that the yellow pigments may play some direct part in the photosynthetic reaction. Warburg has measured the quantum efficiency of the reaction and finds that for various wave-lengths of light the efficiency varies. This he puts in table form:

Wave-length in μ	660	578	436
Quanta/moles of O_2	4.4	4.3	5.1

When he measured the absorption of light by the yellow pigments he found that in the blue they absorbed 30 per cent of the light. Therefore, if only the light absorbed by chlorophyll were active, 70 per cent of the number of quanta should be used in this process, i.e., $.7 \times 5.1$ or 3.6 quanta per molecule of oxygen liberated. This, however, gives too high a quantum efficiency when compared with the other parts of the spectrum and he says:

The variation [i.e., in the yield] in the blue, though not very considerable, yet far outside the limits of error, we will explain as follows: In the red and yellow only the chlorophyll absorbs, in the blue besides the chlorophyll the yellow pigments also absorb. One can now well assume that the primary processes in the red and yellow are the same, but it is true that the primary processes in the blue are in part other than in the red and yellow. . . . Obviously the energy absorbed by the yellow pigments is active, but with poorer yield, than the energy absorbed by the chlorophyll.

Thus we see that almost every conceivable physiological function has been ascribed to the yellow pigments and yet not one clearly demonstrated. Indeed it is impossible to go farther until the chemistry of these pigments is better understood than it is at present.

Realizing that a thorough understanding of the chemistry of carotene and xanthophyll would facilitate the accumulation of knowledge concerning their physiological function, we started work four years ago on their chemical constitution. During this time other laboratories have joined the search, and now it seems probable that in the very near future the structure of these compounds will be made clear.

During the course of investigations on plant pigments a great many colorants have been isolated. A considerable number of these belong to the general class called carotenoids. They have been given this name because of their likeness to carotene in color, solubility, and characteristic color reactions with concentrated acids. Investigations on the simpler members of this group have proved to be of the utmost value in making clear the constitution of the prototype of the group. For this reason these researches will be detailed first as a foundation upon which to place the structures of carotene and xanthophyll.

In order that we may have in mind certain members of the group they will be listed in tabular form with their empirical formulae and occurrence. The discovery of these has occupied a period of slightly over a century, the first, carotene, having been isolated in 1826, and the last, physalien, in 1929. It is also of interest to note the number of isomeric substances in the group, and it is very probable that a closer examination of these individuals may separate them into a number of isomers.

TABLE OF CAROTENOIDS

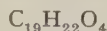
Name	Formula	Occurrence
Carotene	$C_{40}H_{56}$	Leaves; carrot roots
Lycopin	$C_{40}H_{56}$	Fruit of tomato
Xanthophyll	$C_{40}H_{56}O_2$	Green leaves; egg yolk
Zeaxanthin	$C_{40}H_{56}O_2$	<i>Zea mays</i>
Fucoxanthin	$C_{40}H_{54}O_6$	Olive brown seaweeds
Bixin	$C_{25}H_{30}O_4$	<i>Bixa orellana</i>
Beta-Crocetin	$C_{20}H_{24}O_4$	<i>Crocus sativus</i> (Saffron)
Capsanthin	$C_{34}H_{48}O_3$	Paprika
Physalien	$C_{60}H_{96}O_4$	<i>Physalis alkekengi</i> and <i>franchetti</i>

A chronological recitation of facts in support of the structures proposed for various carotenoid pigments would be extremely bewildering, so this will not be attempted. The method of presentation will be, rather, to cite only the material essential to the establishment of a given constitution in as logical a sequence as possible. Only in this way can the meaning of the structure be set forth clearly.

Crocetin.—The first compound of this group which will be discussed is crocetin, the simplest member of the group. The elucidation of its constitution has served in large measure to clear away certain erroneous ideas and to give a hypothesis upon which to base the structure of the remaining members of the series. The discussion of the structure of crocetin first will serve admirably as an introduction to the discussion of the more complex configurations.

Crocetin is prepared (12) by extracting saffron powder *Crocus sativus*, previously freed from fats and oils, with alcohol and allowing the pigments to crystallize from this solvent. Red crystals of three different substances separate from the solution. These substances are very closely related to each other and are called alpha-, beta-, and gamma-crocetin. The empirical formulae assigned these compounds are (12e):

alpha-crocetin



beta-crocetin



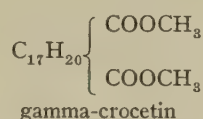
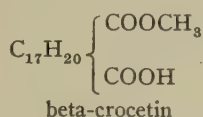
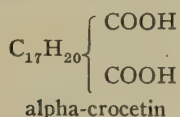
gamma-crocetin



The first of these gave a di-potassium salt (18a) of the formula $C_{19}H_{20}O_4K_2$, which showed that the compound was a dicarboxylic acid.

The beta-crocin formed a mono-potassium salt (12b); and the gamma compound formed no salt at all.

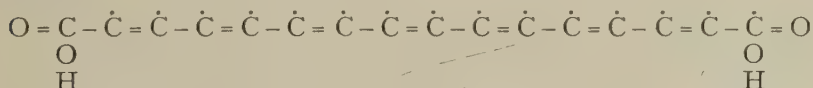
When, however, the three were assayed for CH_3O groups, the first gave none; the beta-derivative, one; and the gamma-crocin, two such groups (12c). Therefore the relationship of the three compounds became evident (12d). The alpha is a dicarboxylic acid, the beta a half-methyl ester, and the gamma-derivative the di-methyl ester. Thus we can formulate the three as follows:



Conversely the alpha- and beta-compounds can be converted into the gamma-crocin by methylation with diazomethane. This then confirmed by synthesis the interrelation between the three crocins.

The next problem was to determine the structure of the $\text{C}_{17}\text{H}_{20}$ group. When the gamma-crocin was hydrogenated catalytically, it was found to absorb 7 mols of hydrogen and to give the compound $\text{C}_{17}\text{H}_{34}(\text{COOCH}_3)_2$ (12f). It is seen that this compound is completely saturated, making it evident that crocin has an open chain skeleton.

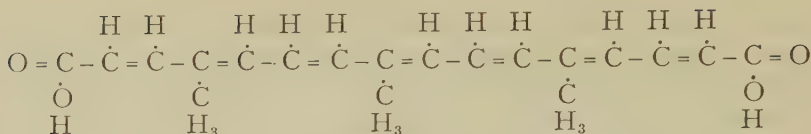
Next it was necessary to determine whether or not the chain is straight or branched. Since the compound is brick-red in color it is assumed that all of the double bonds are conjugated, for, as we shall show later, color is produced by long series of conjugated double bonds. If the double bonds are all conjugated, then alpha-crocin will have the following skeletal structure:



The chain must therefore be branched, for we have three more carbon atoms to allocate.

This allocation of the remaining carbon atoms was solved by Kuhn through the determination of the number and kind of volatile acid molecules gotten by permanganate oxidation. He definitely proved that three moles of acetic acid are thus formed. This shows definitely that the three carbon atoms are present in the molecule as three methyl side chain groups.

Because of the similarity of this compound with other compounds in nature, it was assumed that this molecule was made up of condensed isoprene nuclei, $\text{CH}_2:\text{CH}.\text{C}(\text{CH}_3):\text{CH}_2$. If this be the case, then the distribution of the methyl groups is known, and the structure (12g) will be represented by this formulation:



Alpha-crocin

Further proof of the correctness of this structure came through the determination of the oxygen equivalent (16c) of this compound. The following equation should be valid for the structure assigned.



It was found that on oxidation with permanganate, 33 O atoms were used up and 3 acetic acid molecules formed, thus substantiating the structure proposed.

The structure of crocetin is then established.

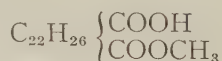
For phytochemistry it is very interesting that in the original plant material this acid is found linked with a disaccharide which has very recently been identified as gentiobiose (11). This may be very significant to those who are interested in yellow pigments and their photosynthetic relationships.

Bixin.—The next carotenoid whose structure has been fairly well established is bixin. This coloring matter occurs in the seeds of *Bixa orellana*, probably combined with a sugar. For a great many years this colorant was used in the artificial coloration of butter and cheese. The method (15b) of preparation of this pigment is very similar to that of crocetin, the raw material being in this case the seeds of the arnatto tree. Bixin separates from the alcoholic extract in deep red crystals.

The facts which have led to the proposed structure for bixin will now be given.

Investigators are not entirely agreed on an empirical formula for this compound. A very recent critical review (10a) of all the available analytical data in the light of the known chemical facts seems to indicate that $\text{C}_{25}\text{H}_{30}\text{O}_4$ is right, and in the present argument this will be assumed to be correct.

Bixin forms a mono-potassium and sodium salt (3, 18b), showing that it is a monobasic acid. On hydrolysis it gives a dibasic acid as is shown from the analysis of its dipotassium salt (15a). Furthermore when it is treated with diazomethane it is esterified, giving a compound of the following formula $\text{C}_{26}\text{H}_{32}\text{O}_4$ (10c). When bixin is assayed for methoxyl groups it is shown to contain one CH_3O group (10b). Thus we find that the oxygens are present entirely as carboxyl oxygens and the structure may be indicated by this formula:

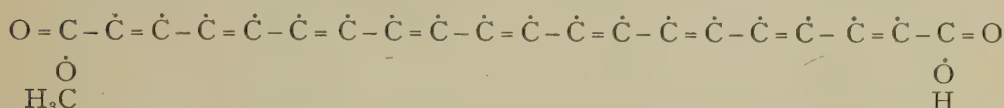


When the methyl ester of bixin is hydrogenated catalytically (8, 18c) it is found to absorb 9 mols of hydrogen, thus producing a compound $C_{26}H_{50}O_4$, whose structure then is



This shows that the hydrocarbon residue has been entirely saturated and is an open chain.

Since the unsaturation in bixin is due entirely to double bonds and since it has such a deep color, it may be assumed, as in the case of crocetin, that these double bonds are conjugated. This then would fix the foundation of the structure of this compound as:



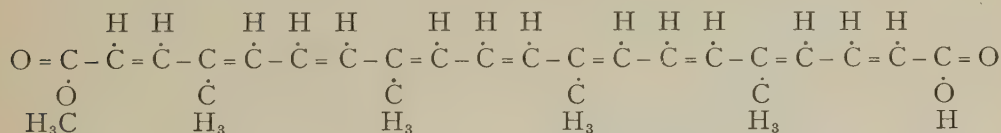
This leaves 4 carbon atoms still to be placed to complete the carbon skeleton of the molecule.

The placement of the carbon atoms was determined in a manner strictly analogous to that of crocetin. On oxidation of bixin with potassium permanganate the following equation (16*b*) was found to be valid:



This showed that the 4 carbon atoms are present in the original molecule as 4 methyl side chains. Furthermore, the oxygen equivalent agrees well with that demanded by the proposed structure.

By ozonization (16a) two compounds were isolated which threw additional light on this structure, viz., methyl glyoxal, $\text{CH}_3\text{CO}\cdot\text{CHO}$, and beta-acetyl acrylic ester, $\text{CH}_3\cdot\text{CO}\cdot\text{CH}:\text{CH}\cdot\text{COOCH}_3$. These products, combined with the theory that the carotenoids are made from condensed isoprene nuclei, led to the following proposed arrangement for bixin:



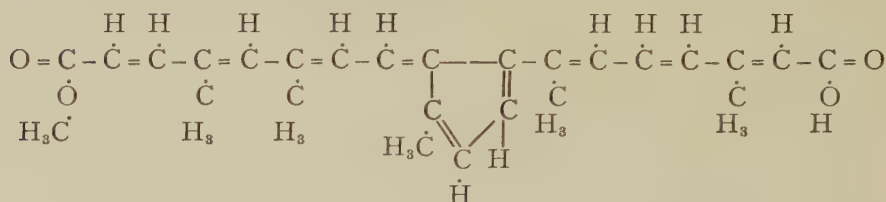
Bixin

Two objections have been raised to this structure. The first is that it does not account for another compound obtained by ozonization,



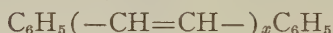
The second objection is that such a structure would not account for the intense color of bixin.

To eliminate these two objections and to make the structural representation agree with the other proposed empirical formula, $C_{26}H_{30}O_4$, Faltis and Viebock (5) have proposed a ring structure:



Such a structure does account for the presence of the compounds observed on ozonization, and would undoubtedly have a very deep color. There are objectionable features, however, which make such a picture improbable. These objections may be summarized as follows: No ring compound has been isolated from the reaction products of bixin; it does not agree with the oxygen equivalent nor the number of acetic acid molecules produced by oxidation with potassium permanganate. We are inclined, therefore, toward the structure proposed by Kuhn.

The argument put forward by Faltis and Viebock that such deep color cannot be produced by a conjugated system of double bonds alone is refuted by the fact that Kuhn (17a) has synthesized a series of compounds which contain only conjugated double bonds as the chromophoric group and which have a depth of color proportional to the number of double bonds in the conjugated system. The general formula for this series is



where x varies from 1 to 8. The following table gives the value of x , the name of the compound, and the observed color. For the sake of a quantitative correlation between the number of double bonds and color, it is unfortunate that absorption spectra measurements are not available.

Value of x	Name	Color
1.....	1,2 diphenyl ethylene	White
2.....	1,4 diphenyl butadiene	Yellow cast
3.....	1,6 diphenyl hexatriene	Light yellow green
4.....	1,8 diphenyl octatetraene	Chrome-yellow with green cast
5.....	1,10 diphenyl decapentaene	Orange
6.....	1,12 diphenyl dodecahexaene	Brown-orange
7.....	1,14 diphenyl tetradeca heptaene	Copper-bronze
8.....	1,16 diphenyl hexadeca octaene	Copper-red with blue tinge

From this table it is evident that increased conjugation shifts the color of the compound toward the red. The color of both crocetin and bixin correspond well with the polyene containing the same number of conjugated double bonds. Assuming that the carboxyl group has the same effect as the phenyl group on color, the number of conjugated double bonds can be esti-

mated in the natural pigments by matching their color with the synthetic products. Thus the color of these plant pigments is explicable on the basis of conjugation alone.

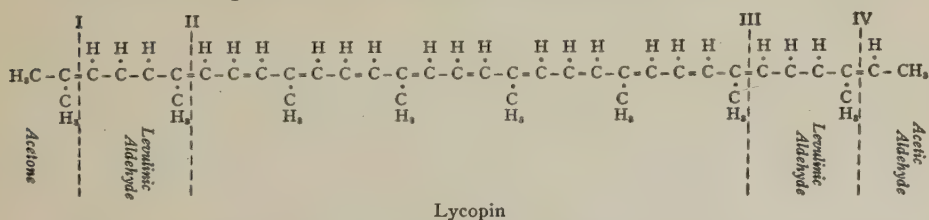
Although the positions of the side chains in the structure for bixin proposed by Kuhn may possibly have to be changed, the picture represents admirably the reactions which the molecule undergoes. The essential features of this structure may be assumed to be definitely fixed.

Lycopin.—Considerable work has been done on the coloring material of the tomato fruit (*Lycopersicum esculentum*). This pigment was first investigated in 1876 by Millardet who obtained it in crystalline condition. At first it was thought to be the same as the previously discovered carotene, but absorption spectrum analysis (21a) showed that the absorption bands of the two compounds were quite different. The true chemical nature of the compound was first obtained through elementary analysis by Montanari, who showed that it had the same composition as carotene. From the later work of Escher (2) the empirical formula was established to be the same as that of carotene. Not much advance was made in the knowledge of the structure until very recently, when Karrer and his co-workers, using methods similar to those described above, have given us essentially the correct picture of the lycopin molecule.

Elementary analysis and molecular weight determinations showed the empirical formula to be $C_{40}H_{56}$. By catalytic hydrogenation lycopin was found to absorb 13 mols of hydrogen per mol of pigment (13). This definitely established the open-chain nature of the product. The perhydrolycopin thus formed had the formula $C_{40}H_{82}$, definitely a saturated hydrocarbon, and free from any ring structure.

Further, lycopin was shown by Pummerer and his associates (21b) to absorb 13 mols of iodine chloride, thus substantiating the work of Karrer as to the number of double bonds in the molecule.

By ozonization, Karrer (9) was able to split the lycopin molecule and identify acetone, acetaldehyde and to get evidence that levulinic aldehyde was formed, although this is still somewhat uncertain. From the data thus obtained he has pictured the molecule in the following way:



Thus, by splitting at position I, acetone is formed; at I and II, levulinic aldehyde is produced; by breaking at IV, acetaldehyde is split off, and by cleaving at IV and III a second molecule of levulinic aldehyde is produced.

The question now arises as to the placement of the double bonds. If all 13 belong to a single conjugated system, that system would have to lie near one end of the chain to allow acetone to be formed on ozonization. This would then leave a saturated portion of the carbon chain at the other end, which on oxidation would appear as one of the aliphatic acids which could be easily identified. No such acid has been found. This points then to the fact that the 13 double bonds do not belong to a single group.

The color of lycopin corresponds to the synthetic diphenyl hexadeca octaene (17c). According to Kuhn, this polyene has the color equivalent to 11 conjugated double bonds, assuming that each phenyl group has the same effect on color as 1.5 double bonds. This then indicates that only 11 of the double bonds in lycopin are conjugated. As to the arrangement of the remaining two, Karrer prefers to make these symmetrical with respect to the conjugated system.

Further work must be done to confirm these assumptions before the structure can be accepted unreservedly.

Carotene.—Carotene was the first member of this group to be isolated and is perhaps the best known of all the pigments of this class. This is evidenced by the fact that the group is called carotenoids, meaning carotene-like. It was first described by Wachenroder in 1826, but was not isolated in sufficient quantity for analysis until 1847, when Zeise showed by analysis the hydrocarbon nature of the pigment. Very little more was added to the chemistry of carotene until Willstaetter and his students undertook its study. They found the correct empirical formula to be $C_{40}H_{56}$ (25a).

No real insight into the nature of the molecule came until Zechmeister and Cholnoky (28a) showed that by catalytic hydrogenation carotene absorbs 11 mols of hydrogen to give the compound $C_{40}H_{78}$. Since they could get no further hydrogenation they assumed that of the 13 possible unsaturated linkages 11 are present as double bonds and two as rings.

These investigators (28b) found that on catalytic hydrogenation the color disappeared and that there was a direct proportionality between the decrease in color and the amount of hydrogen absorbed to the point where 80 per cent of the color had disappeared. By a simple calculation they concluded that all of the color would have been dissipated by the addition of 8 mols of hydrogen. By combining this with the previous observation that a total of 11 mols could be absorbed, they arrived at the conclusion that there are two systems of double bonds in the molecule, one of 8 double bonds, responsible for the color, and another of 3 double bonds, much less reactive and without influence on the color.

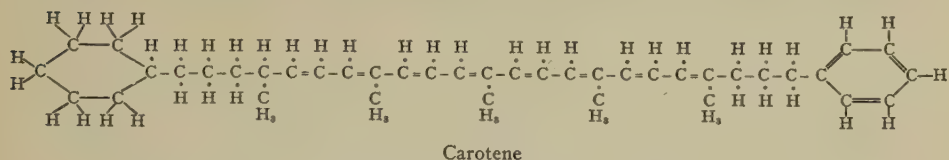
This distribution seemed to be confirmed by the work of Pummerer (20) using benzoyl hydroperoxide as a reagent for active double bonds. With this agent he showed that 8 of the 11 double bonds are active.

Later Zechmeister and Tuzson (29d) showed that carotene reacted

with 8 mols of bromine to give true addition before more deep-seated reaction set in.

Of fundamental importance is the observation that carotene itself has been found to be optically active (23*a*, 14*c*), while the perhydro-carotene is optically inactive (29*b*, 28*c*).

To account for all of these facts and also to keep the representation of carotene in harmony with the structures of the other pigments of this group, Zechmeister (17*b*) has proposed the following structural formula:



It accounts very nicely for the distribution of double bonds into two groups of 8 and 3 each; for the optical activity of carotene and inactivity of perhydro-carotene and partially for the isoprene theory of construction of these compounds.

On the other hand, there are some very definite objections to this picture of the molecule. In the first place, benzoic acid has never been found as an oxidation product of the carotene molecule, although several attempts have been made to isolate this derivative. In the second place, if we assume a structure analogous to the known structures of this class, we would expect the molecule to be built of isoprene units throughout, which would produce cymene and hydrogenated cymene derivatives instead of giving benzene and reduced benzene nuclei. Furthermore, as Kuhn points out (17*b*), such a system of 8 double bonds, isolated from a benzene nucleus, would not account for the deep red color of carotene. Such a system would correspond to 1,10 diphenyl deca-pentaene which possesses only an orange color.

More recent work shows that, instead of a division of the 11 double bonds into one group of 8 and another of 3, they should be divided into groups of 9 and 2, respectively. This is borne out by two sets of experimental facts.

To establish the theory of Zechmeister and Chohnoky that the number of double bonds in a conjugated system can be calculated from the change of color on hydrogenation, Zechmeister and Vrabély (30) followed this change on a substance known to contain 9 conjugated double bonds, bixin. They found that the theory was erroneous because they accounted for only 8 of the 9 double bonds in this way. However, they did establish the fact that carotene and bixin reacted similarly to the point where 9 mols of hydrogen had been added. From this they concluded that carotene possesses a conjugated system of 9 rather than 8 double bonds.

A further experimental proof of the correctness of this idea came from the catalytic hydrogenation of carotene in this laboratory (23c). The conditions employed by Zechmeister and Cholnoky were changed so that only a small amount of platinum oxide catalyst relative to the amount of carotene was used. A different solvent also was employed. In this case only 9 mols of hydrogen were absorbed and gave a compound whose composition agreed with the formula $C_{40}H_{74}$. No further hydrogenation could be induced by longer shaking with the catalyst. This very definitely set 9 of the double bonds apart from the others.

The fact that carotene adds only 8 moles of halogen (29d) and reacts with only 8 mols of benzoyl hydroperoxide does not necessarily indicate that there are 8 double bonds in the conjugated system, for experiments with other conjugated systems show that they do not attack all of the double bonds in the system. For example, in bixin with 9 conjugated double bonds only 6 can be accounted for by these reactions (21c).

It seems likely, then, that there is a system of 9 conjugated double bonds in carotene.

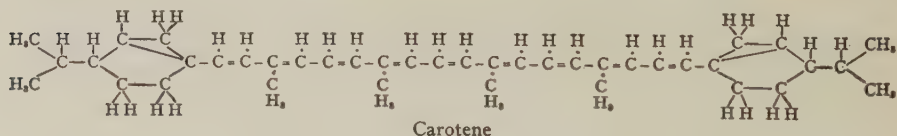
The octa-deca-hydro carotene was isolated and its physical properties studied. Instead of being optically inactive as is the compound $C_{40}H_{78}$, it is decidedly dextro-rotatory, which shows that partial hydrogenation does not form an optically symmetrical molecule.

The molecular refraction, which has been used so effectively in the determination and confirmation of structure, was also determined by the Lorenz-Lorentz formula and found to be 180.3 (23c). Calculating the theoretical values for $C_{40}H_{74}$ under various assumptions, the following values were obtained:

Containing four double bonds.....	185.25
Containing two double bonds and two six-rings....	181.98
Containing two thujane or carone rings.....	180.30

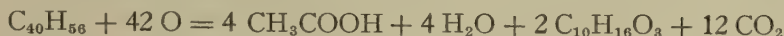
From these data it seems probable that carotene contains two bi-cyclic rings of the thujane or carane type.

Assuming that there are 9 conjugated double bonds, attached to two thujane rings, we may represent the carotene molecule by the following structure:



Additional evidence in favor of this structure was obtained by the oxidation of carotene with potassium permanganate in pyridine solution. When one mol of carotene was oxidized in this way it was found that

exactly four mols of acetic acid were formed. This agrees perfectly with the proposed structure. The oxygen equivalent determined also with potassium permanganate in pyridine showed that 42 atoms of oxygen were used. This agrees well with the theoretical values that would be predicted from the following reaction:



The compound $\text{C}_{10}\text{H}_{16}\text{O}_3$ has never been identified, but a liquid has been isolated from the reaction of carotene, dissolved in petroleum ether, with atmospheric oxygen, which gave analyses for this empirical formula (23*b*).

This proposed structure explains, in addition to the facts just mentioned, the optical rotation of the compound, the optical rotation of the addition product formed with 18 hydrogen atoms, and the symmetrical formula for the completely reduced molecule. The distribution of double bonds into a group of 9 and one of 2 may be seen to have its counterpart in the 9 double bonds and the 2 three-rings. Furthermore such a structure would undoubtedly give a color as deep as that of carotene. Further work is necessary either to validate or invalidate certain assumptions made in the proposal of this picture, but such a structure accounts for the reactions known at the present time.

Xanthophyll.—Almost as many facts have been gathered concerning the structure of xanthophyll as concerning carotene, and yet it is difficult to explain these facts by a graphic formula. The structure in the main must be very similar to carotene. As the empirical formula $\text{C}_{40}\text{H}_{56}\text{O}_2$ shows, however, there are two oxygen atoms in the xanthophyll molecule whose function and position have to be determined. As yet their chemical nature is undetermined. It cannot be said whether they are alcohol, ester, ether, or peroxide oxygens. The work of Willstaetter (25*b*) seems to point to an ether type of linkage. It will take considerable more evidence to establish the correct function of these atoms in the molecule.

The facts as we know them concerning xanthophyll are as follows: Xanthophyll is hydrogenated in a manner entirely analogous to carotene. The molecule absorbs 11 mols of hydrogen (29*a*). The color change with addition of hydrogen follows the same course also. Addition of bromine shows 8 double bonds (29*d*) to be active toward this reagent. Benzoyl hydroperoxide also accounts for 8 double bonds (21*c*). Therefore, so far as the double bonds in xanthophyll are concerned, they seem to be identical with carotene.

An assay of the number of volatile acid residues from xanthophyll on oxidation also points to the same relationship as that observed in carotene, for four molecules of acetic acid have been formed from a mol of the pigment.

The colorant itself is optically active and so are all of its hydrogenation

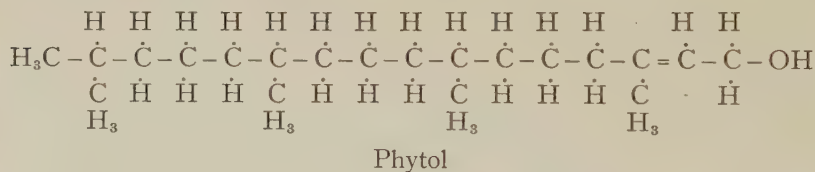
products (29c). This points to a placement of the oxygens in such a position that they will make the perhydro-derivative (as the addition product of xanthophyll and 11 moles of hydrogen is called) unsymmetrical as well as the original molecule.

The oxygen equivalent of xanthophyll is much higher than that of carotene. Determined in the same manner, xanthophyll shows a value of 53.3, as against carotene of 42. Since the oxygen equivalent is so much higher than that of carotene, the xanthophyll molecule is either very dissimilar to that of carotene, or the positions of the oxygens give an added point of attack for oxidation. From the similarity in reactions and the absorption spectra curves it seems logical to suppose that carotene and xanthophyll are very much alike. It is probable that the oxygens in xanthophyll will be found to have a cyclic oxide structure in the ring portion of the carotene molecule. Work is now in progress which may throw light on this assumption.

The other carotenoid pigments, whose names and formulae were listed in the introduction, have not been investigated sufficiently to warrant discussion here. An elucidation of their constitutions would undoubtedly clarify considerably both the chemistry and the physiological functions of the group.

In conclusion I cannot pass without mentioning a possible explanation of the close relationship between chlorophyll and the yellow pigments existing within the green leaf.

Very recently the chemical structure of phytol (6), the alcohol component of the chlorophyll molecule, has been definitely established and is represented by this formula:



A comparison of this structure with that of the carotenoids obviously reveals a striking similarity. Phytol chemically is a hydrogenated member of the carotenoid group. It therefore seems probable that phytol either comes from the carotenoids, forms the carotenoids, or is produced from the same parent substance. In this way the constant proportionality existent between the yellow and green pigments of the leaf may be explained.

The recent work on the structure of the carotenoids has been most encouraging. The structural relationships of the members of this group seem to be well understood and in the very near future we may expect this century-old problem to be solved.

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NOTE.—Because of the economy of space effected in eliminating the full title of the many articles referred to in this paper, the editor and the author agreed that the form used in this bibliography should be followed. (J. H. C. S.)

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XIII. PHOTOSYNTHESIS OF BACTERIA

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It was Lavoisier who made the fundamental discovery of the importance of atmospheric oxygen in life processes. As no living organism was known which could live without oxygen, he naturally concluded that O_2 was, without exception, necessary for the sustenance of life; metabolism was looked upon as a process of "slow combustion" of organic matter. This point of view held until Pasteur, in studying the microscopic living beings, discovered organisms which were able to live and to develop in the complete absence of free O_2 and formulated his genial conception: "*La fermentation c'est la vie sans air.*" Here was a substitute for the "slow combustion"; here were processes which evolved in the total absence of one of the integral factors of any combustion.

Yet there should be something in common in these two widely divergent phenomena of living matter; the "slow combustion" as well as the "fermentation" would appear to represent merely two different aspects of this common feature. Especially the work and clear insight of Max Rubner made us familiar with the idea that the most important function of both was the liberation of energy. It was this same idea of the function of organic matter as a source of energy for living organisms which, as early as 1887, led Winogradsky (15, 16) to his brilliant conception of the function of inorganic substances as the combustible matter or source of energy in the metabolism of lower organisms.

This conception was based on Winogradsky's experiments on the sulphur bacteria and has been proved conclusively, as far as the colorless forms are concerned, by Keil (8) in 1912. Among the sulphur bacteria studied by Winogradsky were also those forms which had been known, since the time of Ray Lankester, Cohn, Warming, etc., as purple bacteria, characterized by purple-red colored cells. He showed that these organisms also converted hydrogen sulphide into sulphates and, therefore, concluded that their metabolism was similar. Now these purple bacteria behave like true anaërobes, and for the oxidation process would need O_2 . Winogradsky's observations on the favorable influence of minute green organisms (green bacteria) led him to the hypothesis that these latter provided it.

As early as 1882, Engelmann (5, 6) had made the important observation that the purple bacteria were extremely sensitive to light, and therefore put forward the idea that they were capable of converting CO_2 in the light with the liberation of oxygen—a theory which he believed he had

proved in 1887. By the use of Engelmann's motile bacteria method, Ewart (7) and Buder (4) later confirmed Engelmann's results. All attempts, however, to demonstrate the liberation of oxygen by methods other than Engelmann's failed.

Skene (13), after having proved in 1914 that these organisms can grow in a purely mineral medium containing H_2S under almost anaërobic conditions, went back to Winogradsky's hypothesis as to the function of the green bacteria, but Bavendamm (3) made this appear highly improbable on the basis of his experimental results with cultures which, according to him, did not contain green bacteria. This situation is most clearly and critically discussed in Buder's excellent paper on the biology of the bacteriopurpurin and the purple bacteria, which was published in 1919. Therein Buder comes to the following conclusions:

The difficulty which at the present time exists in trying to explain the metabolism of the *Thiorhodaceae* seems to be based on the fact that one is inclined to ascribe an assimilatory function to the pigment according to Engelmann; whereas, on the other hand, we have to consider with Winogradsky the oxidation of the H_2S as a fundamental link of their metabolism—the more so because Skene's experiments and the analogy with the colorless sulphur bacteria also support this. Thus we would then have two sources of energy for the reduction of the CO_2 , the light, and the chemical energy liberated during the oxidation of the H_2S . Up to now one seems to have been afraid to assume the existence of photo- and chemosynthesis independently side by side. From an energetic viewpoint I cannot see any difficulty in the way of this assumption. . . .

Buder then points out that the assumption of a photosynthetic activity is almost a necessary consequence of the observed facts; these organisms, however, in contradistinction to the green plants, would use the oxygen liberated for the oxidation of H_2S .

Yet there remains one great difficulty in the way of this, as Buder calls it, well-founded assumption. Buder discusses in detail the possibility of a purely chemosynthetic activity, and sees the superiority of the purple bacteria over the colorless forms in the fact that the former under anaërobic conditions can provide themselves with the necessary oxygen by a photosynthetic decomposition of CO_2 . Bavendamm, following Buder's ideas, states:

As the red sulphur bacteria can develop in the absence of oxygen and require CO_2 , but also need oxygen for the oxidation of the H_2S , we must assume that the pigments play a rôle similar to the chlorophyll, i.e., that the purple sulphur bacteria can assimilate CO_2 in the light with a simultaneous liberation of oxygen. . . . Thus they can also live during certain periods in places with little or no oxygen, which is impossible for the colorless forms. . . . When H_2S is lacking—under which conditions the colorless forms must die—the red forms still have the other source of energy at their disposition.

And yet, on the next page, Bavendamm states very definitely:

For both groups, the colorless and the purple sulphur bacteria, the H_2S is absolutely necessary.

To my mind the problem of the metabolism of the purple sulphur bacteria lies right here. We must account for the fact that they cannot live except in the presence of both H_2S and light. It is true that from an energetic point of view there is no objection to the assumption that a photo- and chemosynthetic mode of life might exist side by side independently. This would mean, however, that the organisms, once being capable of assimilating (reducing) CO_2 by means of photosynthesis, should be able, also, to develop in a medium free from H_2S .

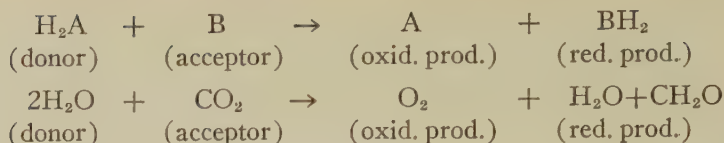
Obviously, this is not true, and there seems to be only one possible explanation of it. It might be assumed that only part of the necessary CO_2 could be reduced by photosynthetic activity, and that the chemosynthetic reaction would have to furnish the additional supply. But if we calculate, on the basis of Winogradsky's data for the colorless sulphur bacteria, the quantity of CO_2 which can be reduced chemosynthetically with the aid of photosynthetically liberated oxygen, we find that 80 molecules of CO_2 must be reduced photosynthetically in order to furnish the necessary oxygen for the chemosynthetic reduction of only one molecule of CO_2 . Quantitatively, then, it appears that there is no necessity for the existence of a chemosynthetic reaction. In fact it would be absurd to assume that this chemosynthetic reaction—made possible only by a previous photosynthetic production of oxygen—would provide the necessary energy for the reduction of part of the CO_2 which, for some obscure reason, could not be reduced photosynthetically.

Hence, the function of the H_2S as an independent source of energy having become highly improbable, we must find another explanation for the fact that there is, apparently, no photosynthetic activity in the absence of this compound.

What is photosynthesis? It has long been known that during photosynthesis a reaction takes place which may, schematically, be represented by the formula



Thus we may say that it represents a typical oxidation-reduction process. CO_2 is reduced, H_2O is oxidized. It was Thunberg (14) who first applied to this process the theory of oxidation and reduction phenomena developed by H. Wieland. According to Wieland's concept all oxidation reactions involve the transference of hydrogen from one molecule, the donor, to another compound, the acceptor. Thunberg pointed out that the reduction of the CO_2 during photosynthesis took place according to Wieland's general scheme, in other words, the CO_2 would act as the hydrogen acceptor, the H_2O as hydrogen donor, and that, consequently, the oxygen developed must be regarded as dehydrogenated H_2O .



A survey of the large number of oxidation-reduction reactions known to be accomplished by micro-organisms led Kluyver and Donker (9) to their theory of the "unity in biochemistry" based upon Wieland's ideas and on Boeseken's dislocation theory of catalytic action. This theory may be briefly formulated as follows:

Each special organism can bring about a specific activation of certain hydrogen atoms in the substratum. When the activation has reached a certain point, this activated hydrogen can be transferred to an acceptor, which, in its turn, is also activated.

The value of this theory as a working hypothesis has been shown by many important contributions in the field of microbial metabolism. It allows, here, a tentative explanation of the metabolism of the purple sulphur bacteria. For if we consider carbon dioxide assimilation (or reduction) in the light of this concept, it follows that, during the well-known photosynthetic reactions of the green plants, the hydrogen of the water molecule is sufficiently activated to be transferred to the carbon dioxide. And it is equally conceivable that other organisms can bring about the activation of hydrogen atoms to the same degree only when they act upon certain other hydrogen compounds.

In this connection attention may be directed to the oxidation of the dextrose molecule by various micro-organisms, all with oxygen as the hydrogen-acceptor. Alsberg (1) has shown that *B. Savastanoi* converts this sugar into gluconic acid only. But Kluyver and de Leeuw (10) have proved that *Acetobacter suboxydans* oxidizes both dextrose and gluconic acid into oxygluconic acid. We may say, therefore, that *B. Savastanoi* can accomplish the first step but cannot go farther and activate the hydrogen of the gluconic acid sufficiently to cause its transfer to the acceptor. *Acetobacter suboxydans*, on the other hand, can accomplish this but is unable to activate the oxygluconate-hydrogen sufficiently, although a great many microbes are known which are capable of oxidizing the dextrose or the oxygluconic acid still further.

For the process of photosynthetic carbon dioxide assimilation (or reduction) we may then say that the reaction

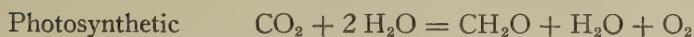
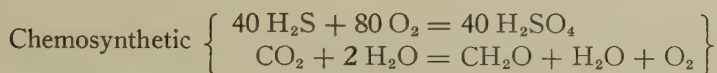


requires various and special compounds of hydrogen for various and special organisms. From which it follows that the photosynthetic activity of the chlorophyll-bearing organisms, in which H_2A represents H_2O , rep-

resents only one very special instance of a group of possible photosynthetic activities. This would mean that the purple sulphur bacteria can use H_2S as a hydrogen donor but cannot use H_2O .

I wish now to show that the results obtained are in complete agreement with this hypothesis.

If, in the case of the purple bacteria, chemosynthetic and photosynthetic activity occurred side by side, we would have the following reactions going on:



These processes being independent of each other, there would not be any quantitative relationship between the end-products H_2SO_4 , O_2 , and reduced CO_2 . But if, on the other hand, the H_2S is regarded as the hydrogen donor for the reduction of CO_2 in the photosynthetic process, according to the equation:



or



a very definite quantitative relationship between the reaction products—reduced CO_2 and S or H_2SO_4 —is required, and the fact that these organisms cannot live except in the presence of H_2S is also explained. The results of a quantitative study of this relationship, with pure cultures, are given in the following tables. Details as to methods of cultivation or determination will not be included.

THIOSPIRILLUM SPEC. 1. (ECTOTHIOFORM, BECKING [2])

All figures represent mg. per 100 cc.

	H_2S Oxidized	H_2SO_4 Produced		CO_2 Reduced	
		Calculated	Found	Calculated	Found
Exp. 1. Analyzed after 14 days...	12.7	36.7	35.8	33.0	32.1
Exp. 2. Analyzed after 24 days...	24.8	71.5	70.9	64.2	63.8
Exp. 3. Analyzed after 25 days...	24.8	71.5	70.2	64.2	63.1
Exp. 4. Analyzed after 47 days...	24.8	71.5	71.4	64.2	63.9

CHROMATIUM SPEC. 3. (ENDOTHIOFORM, BECKING [2])

	H ₂ S Oxidized	H ₂ SO ₄ Produced		CO ₂ Reduced	
		Calculated	Found	Calculated	Found
Exp. 1. Analyzed after 27 days...	8.8	25.5	24.7	22.9	20.7
Exp. 2. Analyzed after 34 days...	17.2*	49.6	43.3†	44.5	39.2
Exp. 3. Analyzed after 42 days...	18.7	53.9	51.4†	48.4	46.8
Exp. 4. Analyzed after 48 days...	18.7	53.9	53.4	48.4	48.9

* Not all of the H₂S used.

† SO₂ = below calculated amount: cells still contain sulphur-droplets.

CHLOROBIVM LIMICOLA NADSON (GREEN BACTERIA) (12)

	H ₂ S Oxidized	H ₂ SO ₄ Produced		CO ₂ Reduced	
		Calculated	Found	Calculated	Found
Exp. 1. Analyzed after 14 days...	26.2	75.4	No soluble	16.9	18.8
Exp. 2. Analyzed after 17 days...	26.2	75.4	sulphur	16.9	17.2
Exp. 3. Analyzed after 30 days...	46.9	135.2	compounds	30.4	28.7
Exp. 4. Analyzed after 36 days...	46.9	135.2	other than H ₂ S	30.4	31.0

Examination of these tables will show, first of all, that, within the limits of error, in not a single instance was the amount of CO₂ reduced greater than the calculated amount. This, in itself, might prove that a CO₂ assimilation in the conventional sense does not take place, especially when it is considered (1) that all of the cultures were continuously illuminated, thus ruling out any possibility that the amount of CO₂ assimilated might equal the amount "respired"; and (2) that the cultures were analyzed after different periods, so that, if the first analysis showed complete oxidation of the H₂S, the cultures later examined would, with certainty, have been "growing" in the absence of any compound which could have been oxidized with the O₂ liberated by the CO₂-reduction process. In this respect the first series is of interest. After 23 days a fresh supply of Na₂S solution was added to the cultures 2, 3, and 4. After 24 hours only, this quantity was completely oxidized; and even after twenty-four days the quantity of CO₂ reduced was exactly the same as that after only one day.

The second series is of interest in that it shows that when the H₂S is not yet completely oxidized (Exps. 2 and 3), as can be determined from a comparison of the quantity of H₂SO₄ produced and calculated as well as by microscopic examination revealing sulphur globules in the cells, the quantity of CO₂ reduced also remains below the calculated amount.

The third series, carried out with a pure culture of green bacteria, shows further that here we deal with organisms which entirely lack the power to dehydrogenate the first oxidation product of the H₂S, the sulphur. It was not possible to make accurate sulphur determinations, since repre-

sentative samples cannot be taken owing to the slimy consistency of the bacterial development. However, the determined amount of reduced CO_2 checks so well with the amount calculated, on the basis of the equation



that there can be little doubt that this is, actually, the process occurring. The fact that these cultures do not contain any trace of soluble sulphur compounds also supports this statement. It is true that in this case some doubt might arise as to whether the sulphur production might not be a secondary process, caused by the oxygen liberated during a "normal" photosynthesis. However, the analogy between these bacteria and the true purples, the fact that the CO_2 reduced is *never greater than that calculated* on the basis of the foregoing equation, together with the fact that they do not develop at all when H_2S is absent, so strongly supports the idea that this, too, is an "abnormal" photosynthetic process, i.e., one in which other hydrogen donors take the place of H_2O , that it seems entirely justifiable to conclude that this equation actually represents the metabolism of the green bacteria.

Consequently we have at present the experimental evidence of the existence of at least two photosynthetic processes which are fundamentally different from that of the green plants. But there is more. When we direct our attention to that group of organisms which has been called "Athiorhodaceae," we find microbes that apparently require organic substances. But what can we say here about the function and influence of light?

It is interesting to consider the few facts which, up to the present, have been established. The observation that these organisms, wherever found in crude cultures, behave like true anaërobes and seem to develop only in the light, plus the knowledge that they certainly can grow under aërobic conditions and in the dark (Molisch, 11), led me to compare their development in the light and in the dark under both aërobic and anaërobic conditions.

Here the fact was established that, whereas under aërobic conditions the cultures seemed not to be influenced by light, the anaërobic cultures developed *in the presence of light only*. We may therefore conclude that the acceptor-action of the oxygen can be substituted for by some other compound only when illuminated. Is this the CO_2 ?

In the present status of this problem the available facts do not yet allow of this conclusion. The difficulty presented by this group of purple bacteria, of which only a limited number of representatives is known, is that, up to the present time, we have not yet been able to obtain a good development in media which can be analyzed afterward. The organisms grow abundantly only in media containing complex organic N-compounds, such

as peptone, yeast autolysate, etc., so that a complete picture of the changes taking place during development can be obtained only after analyses which, for the present at least, seem impossible. They do not develop in media containing H_2S only. They need organic matter, and when small quantities of H_2S are added to the organic media they are always quantitatively recoverable afterward. Therefore, here too the liberation of oxygen by an ordinary photosynthetic activity is out of the question.

What particular influence the light may have upon the components of the medium, which become available as hydrogen acceptors only when illuminated, remains an open question. However, it is probable that the continued study of the metabolism of this group will give additional information with regard to the further possibilities of photosynthesis.

At present we are more or less aware of some of the factors influencing and even governing photosynthesis, especially in so far as they pertain to conditions outside of the cell. We also know something about the inner factors. But from the data available it is still impossible to decide which ones of these factors are really indispensable. We have nothing but hypotheses and theories.

And here, especially, lies the importance of the study of these "abnormal" photosynthetic processes, because a comparison of the factors and conditions which are required for their accomplishment will enable us to find those characteristics which are common to all. It will then be possible to derive the fundamental laws underlying all photosynthetic processes and to correlate these into a general view.

In considering photosynthesis as a process in which CO_2 is reduced by hydrogen from some compound H_2A , it must be kept in mind that this reaction requires the activation of the CO_2 as well as that of the H_2A . And then it must be possible to find that hydrogen compound which requires either no activation at all (H_2S in the case of the green bacteria?) because it is, in itself, active enough, or which requires the same activation as the CO_2 . In such a case it might become possible to carry out photosynthesis with only one catalyst, which has an oxidation-reduction potential suitable for this activation, and, therefore, to carry out photosynthesis independent of the living cell.

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XIV. THE INVESTIGATION OF THE PIGMENTS OF THE LIVING PHOTOSYNTHETIC CELL

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Pigments have always been attractive to man, and the pigments of plant cells are no exception. During the last hundred or more years a great deal of attention has been given to the extraction and analysis of such pigments, and, in the case of the more important ones, much knowledge as to their chemical composition and properties has accumulated. When, however, we search for information concerning the chemical nature and physical state of these same pigments as they exist in the living cell, we find little or no information about most of them, practically nothing which can be accepted as conclusive about any of them, not even about the green pigments which have occupied so much of our attention. It is the purpose of this present article briefly to summarize this knowledge, to discuss the methods by which it has been obtained and may be extended, and to emphasize the importance of so doing.

The very interest in these pigments, the endeavor to isolate them in a "pure" state, is, perhaps, in a large measure responsible for this lack of knowledge of the pigments in their natural state. The extraction and preparation of pure pigments has, naturally, been mainly the concern of organic chemists, and there has always been a tendency to regard such preparations as the "true" pigment. In earlier days it was almost automatically assumed that the preparation, however much man-handled, existed in the plant cell in that identical form. Later when it became evident that there were marked chemical and physical differences between some of these preparations and their predecessors in the plant cell, there was still a tendency to regard the cell pigment as a slightly chemically or physically altered form of the isolated pigment, and to gloss over the differences. This point of view has led to numerous attempts to make preparations of the extracted pigment which would have all of the properties characteristic of the native pigment; but, while much valuable information has been gained in the process, none of them have, so far, succeeded. Most of the experimental work of this type has been carried on with the green pigment, chlorophyll, extracted from leaves, which will be discussed later in this paper.

In contrast to this attitude we have the more biologically sound one of regarding the cell pigment as the primary substance, whose characteristics are to be determined with the maximum attainable accuracy, the extracted pigments being of secondary importance. It is the major contention of this paper that, with the methods which are now available, or which can

readily be developed in the light of our present knowledge, it will not only be possible to determine with accuracy, and to analyze, the absorption spectrum of the living cell, but to arrive at many new conclusions in regard to the chemical and physical nature of the pigments as they exist *in vivo*.

Much has been learned about photosynthesis by analyzing the input and output of the plant under varying conditions, but such methods can only indicate the nature of the process. If we are to understand the chemical details of photosynthesis it would seem necessary to have a fairly accurate knowledge of the components of the system in which it takes place. This knowledge is still to a large degree lacking, and at no point is the lack more conspicuous than when we come to the photodynamically active pigments, which apparently catalyze the whole reaction. Every increase in our knowledge of this reaction system brings nearer the possibility of setting up a model which will absorb radiant energy and convert carbon dioxide and water into a carbohydrate, or into something that we can recognize as convertible into one.

The accurate spectral analysis of the living cell will enable much progress to be made in the study of the photodynamics of photosynthesis, and of the real effects of radiant energy of different wave-lengths, as distinguished from the apparent effects due, only too frequently, solely to differences in the amount of energy absorbed. It will go far toward clearing up the disputed points in the chromatic adaptation of algae to environments where the spectral energy distribution is different from that of normal daylight. It should promptly determine the validity of the contention of Lubimenko (28) that there are at least four groups of green plants which differ characteristically from each other in their absorption spectra, and it may bring to light previously unsuspected relationships or differences of taxonomic interest.

In addition to the already mentioned comparison of the absorption spectra of various preparations of the extracted pigments with the spectrum of the living cell, several other methods have been employed in the attempt to determine the nature of the pigments *in vivo*. Their solubility reactions in the fresh state have been compared with those after heating, drying, or other treatment, and with those of the extracted pigment. Similar comparisons have been made of the photostability of the pigments and of their resistance to acid. Direct microscopic study has also been made of the effects of heat, cold, and various reagents on the pigments in the cell. The fluorescence of the cell pigments has been studied and compared to that of preparations of extracts, and the effect of heat on the fluorescence, and on the fluorescence and absorption spectra, has been investigated to a moderate extent. These diverse methods have led to quite variant conclusions on the part of different investigators, and these will

be mentioned briefly when we come to the discussion of the pigments. In the case of most of the pigments, the experimental study has been extremely limited.

An extended consideration of the results obtained by the above-mentioned methods can but lead one to the conclusion that what is urgently needed is not only the biological point of view suggested above but also, in most cases, a very considerable increase in the sensitiveness and accuracy of the experimental technique. This is nowhere more evident than in the study of the spectral characteristics of the pigments.

Much painstaking work has been done with apparatus wholly unsuited to the purpose. Even with the most careful spectroscopy we cannot determine the maxima and minima in organic band spectra with any accuracy, nor can the relative intensities of absorption in different parts of the spectrum be more than crudely estimated. The method is, furthermore, wholly subjective, and the results are limited, and often distorted, by the visual peculiarities of the observer. The utility of spectroscopy is therefore limited to preliminary observations. The spectrophotographic method is even more deceptive than spectroscopy owing to the varying sensitivity of the plate at different wave-lengths. This objection, of course, applies to a curve plotted from a densitometer record of the spectrophotographic negative as much as to a direct visual inspection of the negative. While the method cannot be used for the determination of the true positions of maxima and minima, it may be of much service in the qualitative observation of spectral changes.

It is evident that the only advantageous general method is spectrophotometry. Here again there are many difficulties to be considered. The preparations which will have to be examined are leaves, thalli, smears, and turbid suspensions, which badly scatter the incident light, and at the absorption maxima the transmission is frequently very low. These characteristics make visual spectrophotometry difficult, as the slit width cannot be increased to a point where sufficient brightness is obtained without the resulting errors becoming serious. The method also suffers to a considerable degree from its subjectivity, and is, of course, only available in the visible. There is a definite limitation to the intensity of the illumination which can be used and to the length of exposure, as the material is both photolabile and thermolabile, and this fact also militates against the sector or polarization photographic spectrophotometer because of the length of time required to get the record. The difficulties arising from the above-mentioned poor transmission qualities of the experimental material would also eliminate all thermocouple instruments from consideration for use in the visible, although a large-aperture recording instrument of this type would be the choice for work in the infra-red region.

This now leaves for consideration only two methods—photographic

spectrophotometry, based on spectrophotography, and the photoelectric-cell spectrophotometer. As a general method photographic spectrophotometry has many advantages. Owing to the integrative action of the photographic plate, it is easy to record accurately the transmission at even the most intense absorption maxima of leaves and of algae, and the analysis of the faint fluorescence spectrum becomes possible. This ability to get along with much lower light intensities immediately permits the employment of narrower slit-widths and of higher dispersion, so that slit-width error is no longer a factor of any importance. The simultaneous recording of intensities over a wide spectral range makes it possible to keep the time of exposure of the experimental material to the light so short that with the proper additional precautions all injury can be avoided. With this method a permanent objective record of an experimental series can be made in a comparatively short time, and the photometric analysis may be made subsequently at the convenience of the investigator. The method can be used in the visible and near ultra-violet with ordinary panchromatic plates, and in the near infra-red with specially sensitized ones, without modification of the apparatus. The main objection to photographic spectrophotometry lies in the fact that the more rapid methods, such as the neutral wedge, do not locate the maxima and minima with sufficient exactitude, while the far more accurate densitometer methods are decidedly time-consuming. It is fortunately true, as pointed out above, that the spectrophotographs can sometimes be used directly when it is only necessary to establish the qualitative fact that the position of an absorption maximum has been altered.

The photoelectric cell spectrophotometer has not yet been developed to the point where it is suitable for general use in the study of the pigments *in vivo*. Instruments with which the readings have to be made by the observer are too slow in operation for use on photolabile material, and the very promising recording type, now being developed by Hardy (12), still requires too large a slit-width for the critical type of work which is now necessary. When, however, such an apparatus can be constructed with sufficient aperture and sensitivity to permit the utilization of its full resolving power, it would seem to be ideal for work in this field in the visible and near ultra-violet, as the experimental data are in shape for immediate interpretation. With the addition of a large-aperture, high dispersion recording infra-red spectrophotometer, the requirements for work on the absorption spectra of leaves, algae, and bacteria would be fully met. For the study of the fluorescence spectrum the photographic method will, however, probably always be required.

Under these circumstances the adoption of photographic spectrophotometric methods was indicated. The instruments and technique which have been developed will be described fully elsewhere. It will be sufficient to

say here that the instruments include a one-meter concave grating spectrograph and a new type of projection comparator-densitometer especially designed for use on organic band spectrum negatives. The spectrograph gives a dispersion of about 15.6 Å per millimeter in the first order, so that on the 10-inch cut film used 3600 Å can be recorded in a single spectrum. The grating is mounted normal to the film, and as the cumulative departure from perfectly linear dispersion is only about 1.5 Å from the center of the negative to either end, the error in treating the spectrum as perfectly normal is of second order magnitude when working with organic band spectra. The practical normality of the spectrum immensely lessens the labor of plotting the photometric measurements. A microscope can be used in the collimating train, as in the apparatus of Becking and Ross (3), or not, according to the nature of the material under investigation. The large aperture of the spectrograph and collimating system permits the securing of negatives with properly exposed absorption maxima in about one minute, with average material and a 6-volt, 106-watt, ribbon-filament lamp as source. The photometric technique employed is a modified form of that developed by Harrison (13, 14) for line intensity measurements.

With apparatus and methods of the above-mentioned type it is now possible to plot the absorption curves and to determine the position of the absorption maxima and minima of the cell pigments with very considerable accuracy. Ordinarily readings are made every 3 mμ, and every 1.5 mμ, near maxima, but this interval can be decreased if necessary. This is to be compared with the more general practice of taking readings every 10 mμ. With this degree of accuracy available it is extremely easy to follow with certitude changes in the absorption spectrum of a substance involving shifts of only a few millimicra in the positions of the maxima and minima. The development of this technique has opened up entirely new possibilities for experimental attack on the problem of the nature and state of the pigments as they exist in the living cell, for the absorption spectrum of a substance is a definite and highly characteristic function of its chemical identity and physical state. We may now establish the normal spectrum of a given cell pigment, and then proceed to attack it in situ with heat, cold, pressure, various chemical reagents, and light of various wave-lengths and intensities. By observing the spectral changes, or lack of change, which may occur under these various conditions, and by correlating them with the known effects of such procedure on various other chemical substances, including preparations of the extracted pigments, it is possible to learn much that never could have been surmised from any amount of study of an extracted pigment which has been chemically altered by the extraction methods used. We have, in effect, transferred our chemical laboratory to the interior of the cell.

Before leaving the question of methods there is one more technique for investigating the absorption spectrum of the living cell which should be mentioned—the various “bacteriogram” methods originated by Engelmann (7, 8). These methods were developed for the study of the relation of photosynthetic activity to spectral absorption. They are based on the observation that motile bacteria will congregate at those points in a varying milieu where a certain peculiar condition exists, and to a degree roughly proportional to the extent to which that particular condition prevails. The method used in studying the relation of photosynthesis to pigment absorption in the green plant will serve as an example. In this case a single filamentous alga was oriented along a spectrum projected in the field of a microscope. The alga was immersed in a culture medium containing oxyphilic bacteria, covered, and sealed. As photosynthesis proceeded the bacteria were observed to gather more thickly along the filament in those places where the absorption was greatest. This distribution of the bacteria was naturally interpreted as being due to the more active liberation of oxygen of photosynthesis at those points, and as indicating that there was a direct correlation between absorption and photosynthesis in all parts of the visible spectrum.

The pigments of every photosynthetic organism should undoubtedly be given intensive study, for an understanding of the differences, as well as of the similarities, of photosynthetic systems may indicate to us which chemical and physical characteristics are fundamental to the process and which are not. It is, however, only natural that man's attention has been more attracted to the pigments of the green plants, so conspicuous in his environment, so vital to his existence. A good measure of our understanding of the exact nature of plant pigments can be had by considering the extent of our present knowledge of the green plastid pigments, studied under the name of chlorophyll for over one hundred years.

The name chlorophyll was originally given by Pelletier and Caven-
tieu (37) in 1818 to the mixture of pigments in the crude alcoholic extracts of green leaves. It has, since then, been applied to a great number of preparations, almost always with the implication, or claim, that they represented a purified form of some pigment existing in the leaf. Reinke (40), in 1884, called attention to the fact that historically the word chlorophyll has been used only to designate the extracted pigments, and, since the classical work of Willstätter (52) and his associates, it would seem better to limit this designation to the green pigments which they have isolated and described. That is to say, by the chlorophylls, or by chlorophyll, we always mean the pure pigments chlorophyll *a*, $C_{55}H_{72}O_5N_4Mg + 1/2H_2O$, and chlorophyll *b*, $C_{55}H_{70}O_6N_4Mg$, mixed in some unspecified proportion, and by “crude chlorophyll” or “leaf extract” we are indicating preparations of the same substances of various degrees of impurity.

The green pigments of the living cell appear, as will be seen presently, to be closely related to the chlorophylls, but to differ rather distinctly from these substances in their chemical and physical characteristics. Partly in order to emphasize this distinction, and partly for manifest convenience, it is proposed to refer to these *in vivo* green pigments as "the phyllochlorins," or simply as "phyllochlorin." This is done without prejudice as to their exact nature or number.

For the earlier investigators there was little reason to suspect that there might be any difference between the extracted and the natural pigments. Stokes (46) had, in 1852, examined the absorption spectrum of the leaf and come to the conclusion that it was the same as that of the extract, which is not surprising when we consider that he made the examination with a very simple prism spectroscope with a candle as the light source. The brilliant red fluorescence of leaf extracts had been known since the work of Brewster (4) in 1834, and it was evident that the leaf, if it fluoresced at all, did so very faintly. Stokes, and later Simmler (42), had observed a weak fluorescence, while other investigators failed to find any, but fluorescence was little understood, and the difference was not regarded as important at the time.

The first indication that there might be a difference between phyllochlorin and chlorophyll to be taken seriously was the observation by Hagenbach (10), in 1870, that the absorption bands of the leaf were distinctly farther to the red than those of the leaf extracts, and this was soon abundantly confirmed. A few years later Hagenbach (11) restudied the fluorescence spectrum of the leaf and was then able not only to observe the leaf fluorescence but also to note that the intensity maximum was displaced toward the red in the same manner as the absorption maximum. Hagenbach also noted that solid chlorophyll, that is to say, highly concentrated preparations such as the residue from an alcoholic leaf extract, do not fluoresce, and in later years this has been confirmed and the observation extended to include aqueous colloidal solutions. Casual observation of laboratory preparations had shown that phyllochlorin was strikingly more photostable than chlorophyll extracts, and attempts to extract the pigments from the leaf with various solvents soon made it clear that their solubilities were markedly different from those of chlorophyll.

Three main lines of interpretation have developed: one in which it is assumed that the leaf pigments are free chlorophyll in some particular state of aggregation or molecular arrangement, another in which they are interpreted as chlorophyll in molecularly dispersed solution in a lipid, and lastly, one in which they are considered to be some sort of compound in which chlorophyll is a prosthetic group. The first and last of these tend to approach each other in their later developments.

The first line of interpretation has led to a large number of attempts

to make preparations of chlorophyll which would have the optical and chemical properties of the leaf. As knowledge of these properties increased this became proportionately difficult. Early attempts, such as Lommel's (27) gelatin "leaves," stained with leaf extracts, and Reinke's (39) solidified paraffin preparations, were later shown to have the absorption spectrum of concentrated molecular solutions of chlorophyll and, besides, failed to correspond well with the leaf in other ways. All of this work, as well as much other, would have been unnecessary if more attention had been paid to the beautiful observations of Sorby (43), made in 1872, in which he is really anticipating the conclusions of Willstätter and Stoll (52, 53). Sorby says:

I find there is every reason to believe that in normal healthy green leaves the chlorophyll is chiefly in a free state. On boiling for a short time in water, the oils and wax present in the leaf combine with the chlorophyll and raise the band somewhat further from the red end . . . when [the chlorophyll is] dissolved in the common fixed oils the band is still further raised.

Iwanowski (17, 18) found that aqueous colloidal solutions prepared by adding water to leaf extracts would, when small amounts of electrolytes were present, give absorption spectra in which the bands corresponded in position with those of the leaf but not in their relative intensities. Herlitzka (15, 16), in 1912, stated that the absorption spectra were identical, and Willstätter and Stoll (52, 53) agreed with him in the contention that the leaf pigments were colloidal chlorophyll, although their spectroscopic results confirmed Iwanowski rather than Herlitzka. In 1913 they stated that the relative intensities of the bands are not quite the same, and in 1918 they printed drawings of the spectra in which this difference is clearly indicated. They found a certain similarity in the solubility reactions of phyllochlorin and colloidal chlorophyll, and also that by adding hydrophilic colloids to the solutions they could greatly increase their photostability and resistance to acids, both of which were otherwise extremely low as compared to the leaf. Wurmser (54), investigating photostability and resistance to acids, and Zirkle (55), as a result of studying solubility, stainability, and reactions with proteolytic enzymes, came to much the same conclusions as Willstätter and Stoll except that they both believed the association of the chlorophyll and the protective colloids to be a somewhat closer one, Wurmser considering that the colloid is adsorbed on the pigment in the form of a protective layer, and Zirkle that the pigment is adsorbed on the colloidal particles of the plastid stroma.

There are a number of objections which may be made to the colloidal chlorophyll hypothesis. In the first place, there is apparently a small but consistent difference between the absorption spectra of such preparations and of the leaf. In the second place, the solubility reactions of the fresh leaf only resemble those of colloidal solutions of chlorophyll under con-

ditions which never exist in the living leaf, that is to say in the absence of electrolytes. It is entirely beside the point to compare the solubilities of dried leaf meal, with a drop or two of water added, to those of colloidal solutions of chlorophyll with a trace of electrolyte added. The third objection is most definite: colloidal solutions of chlorophyll show no fluorescence, while the leaf does.

Noack (33) has recently made an interesting attempt to get around this last objection. He has found that in anhydrous systems he can prepare fluorescent adsorbates of chlorophyll, and concludes that in principle there is no bar to supposing that chlorophyll exists in the leaf in the condition of a fluorescent adsorbate on a protein. He observed that on heating the leaf, or aqueous preparations derived from it, for a few seconds at temperatures between 70° and 100°C., the fluorescence was greatly weakened, or disappeared, but on continued heating in most cases it reappeared with its original or even greater intensity. He offers the ingenious explanation that the first short heating either denatures or coagulates the proteins on which the chlorophyll is adsorbed, so that the chlorophyll is desorbed and is then present in the colloidal state, which is non-fluorescent. Further heating melts the cell lipoids and the chlorophyll then passes into a state of molecular solution in the melted lipoids, in which state it again fluoresces.

There are two basic objections which may be raised against Noack's interpretation. In the first place, he was not able to prepare fluorescent adsorbates in aqueous systems; and the leaf is an aqueous system. The second objection arises from observations made by the author in the course of a preliminary study of the effect of heat on the absorption spectrum of the leaf. This phenomenon, originally noted by Sorby (43) as mentioned above, was studied by Willstätter and Stoll (52, 53), whose observations on the shift of the main absorption band in the red correspond closely with my own if we compare the mid-points of their bands with the spectrophotometric maxima. The shift on heating appears to be from 677.5 m μ to 671 m μ . What has not been previously noted is that this shift is a function of both temperature and time, taking place very rapidly at temperatures above 70°C., and much more slowly at lower temperatures. The times and temperatures where the spectral shift takes place in less than one minute correspond exactly with those at which Noack observed the disappearance of fluorescence to take place. Subsequent heating, which causes a return of the fluorescence, causes no further shift of the absorption spectrum that could be observed. In view of Willstätter's and Stoll's observations of the close correspondence of the absorption spectrum of the heated leaf with that of chlorophyll in solution in lecithin, there is no reason to doubt that the immediate result of heating the leaf is the formation of a solution of chlorophyll in some organic solvent. It is

evident that colloidal chlorophyll is not formed, as in this case the shift of the absorption spectrum should coincide with the reappearance of the fluorescence and not with its disappearance.

Very little need be said about the interpretation of phyllochlorin as chlorophyll in solution in a lipid of high index of refraction. This theory has been held by many; Tschirch (48) upheld it vigorously, as did Stern (44, 45) in more recent years. Its only mainstay has been that it accounts for the fluorescence of the leaf; its inadequacies are manifold. We now know that it is futile to attempt to account for the leaf spectrum on the basis of Kundt's (19, 20) "law" that the higher the index of refraction of the solvent the more the absorption bands of the solute are shifted to longer wave-lengths. Not only is the spectrum of chlorophyll in lipid solution different in type from that of the leaf, but solutions in substances known to be present in the leaf in any notable quantity give spectra corresponding in position to that of the heated rather than to the natural leaf. Stern's own observations of the fluorescence of *Chlorella* and of solutions of chlorophyll in lecithin indicated a difference of 4 $m\mu$ in the red absorption maxima, and such differences can no longer be disregarded. The solubility reactions of chlorophyll in lipid solution correspond only with those of the heated leaf, and not at all with those of the natural one. The same thing applies to both the photostability and the chemical reactions. Noack's (33) observation of the weakening of fluorescence on short heating can be used as a final argument against this point of view. It has been shown, both by Walter (51) and by Merritt (29), that except in very dilute solutions the absolute fluorescence decreases with concentration, and very rapidly at the higher concentrations. It is evidently necessary to Stern's theory that the chlorophyll should be in highly concentrated solution. Heating the leaf can therefore only be assumed to bring about a dilution of the chlorophyll solution, and this should result in an increase of the fluorescence proportional to the dilution and not, under any conceivable circumstances, to a decrease.

The more biological view of phyllochlorin as a chemical substance (or substances) existing in the living leaf has gained adherents slowly. In 1884 Reinke (40) became conscious of the improbability that a system in which the chlorophyll was imbedded in a solid fatty or waxy substance would be able to function in the photosynthesis of carbohydrates from carbon dioxide and water. He then went on to suggest that the chlorophyll was most probably chemically combined with the protoplasmic ground substance of the chromatophore in a union very sensitive to alcohol. More recently, Palladin (36) concluded that the leaf pigment was chlorophyll in combination with a lipid, and Osborne and Wakeman (34), in a paper on the proteins of the spinach leaf, expressed the opinion that there were good grounds for supposing that, in the leaf, chlorophyll was in

some sort of combination with a protein and a phosphatid. In one of Osborne's (35) latest papers he reiterates this view, with particular emphasis on the probability of the existence of a chemical union of chlorophyll and a protein, stable to ether but not to alcohol. Lubimenko (28) maintains that *la chlorophylle naturelle* is an albuminoid substance which varies chemically in the different plants, just as do the other albuminoid substances of the protoplasm. *La chlorophylle naturelle*, or phyllochlorin, is considered by Lubimenko to be an exceedingly comprehensive compound which, when coagulated by boiling, freezing, alcohol or acetone, or otherwise, breaks down into a colorless albuminoid portion and a whole series of green and yellow pigments. It is apparently his contention that while, on the one hand, varying conditions of coagulation or extraction will produce varying series of green and yellow pigments, it is possible, on the other, to obtain a number of different xanthophylls from different plants when the conditions are constant. The first statement is simply a repetition of the whole history of chlorophyll study, and there is a good reason to doubt the second. Tswett (49) found much the same thing, and it has since been shown that several of his xanthophylls are oxidation products; in fact, it is only when the greatest care is taken that their formation can be avoided.

It is too soon to be able to express any very definite opinion as to the exact nature of phyllochlorin on the basis of the author's work. It may, however, be said that there is every indication that it has at least the complexity of a compound of chlorophyll with an albuminoid or a globulin, in a manner perhaps analogous to haem and globin, and that it is closely associated with a lipid, using this word in its broadest sense. Such a compound could be expected to possess the very properties which phyllochlorin has been observed to have. There does not seem to be any strong present reason for assuming that the carotinoid pigments form an integral part of phyllochlorin, and Willstätter and Stoll (53) have shown that they vary in amount independently from chlorophyll, but the possibility is not excluded. If phyllochlorin is of the type of an albumin-chlorophyll compound we could certainly expect it to be at least as photostable and as resistant to acids as colloidal chlorophyll protected by adsorbed albumin, and probably more so. My own observations as well as those of Lubimenko, and even of Willstätter and Stoll, appear to show that chlorophyll can only be extracted from the plant cell by fat solvents under conditions which are known to bring about the denaturation (rather than coagulation) of albumins and globulins, and this would tend to imply the existence of such a compound. Further evidence pointing in this direction is to be found in Noack's observations on the conditions under which the disappearance of fluorescence takes place and in my own observation that the corresponding shift of the absorption spectrum is a

function of both time and temperature. This function appears to be logarithmic and to correspond to those found by Chick and Martin (6) for the denaturation of egg albumin by heat, and by Lepeschkin (25, 26) for the effect of heat on the permeability of the beet-root cell to its contained anthocyanins and for the heat coagulation of proteins. Zirkle's (55) observation of the remarkable photostability of phyllochlorin when dehydrated with glycerine, and of the loss of this stability on the addition of either water or alcohol, points in the same direction.

The greatest difficulty in the way of all theories involving colloidal chlorophyll has been that such preparations do not fluoresce. There are good theoretical reasons for expecting that a compound in which chlorophyll was combined as a prosthetic group with one, or probably more, protein molecules would be able to fluoresce to some extent, and that no preparation of colloidal chlorophyll could ever do so. As pointed out above, the intensity of fluorescence is a function of molecular separation, decreasing rapidly as the fluorescing molecules get closer together and apparently interfere with each other's ability to fluoresce, and terminating in complete extinction. In colloidal aggregates we have the physical condition for the extinction of fluorescence, and it makes no difference what we do in the way of adsorbing them; as long as the aggregates exist there will be no fluorescence. This fact explains Noack's (33) failure to get fluorescent adsorbates from aqueous solutions. It may possibly be that in the leaf we have molecularly dispersed chlorophyll adsorbed on the proteins, but it is difficult to conceive of the origin of such a system and it would seem far more probable that the separation and consequent fluorescence of the chlorophyll groups, as well as the form of the absorption spectrum, was brought about by their being combined with one or more protein molecules. It should further be emphasized that such a concept of phyllochlorin is in every way consistent with the data of photosynthesis and is in harmony with our present knowledge of other physiologically active pigments such as haemoglobin, phycoerythrin, and phycocyanin.

The question of the multiplicity of *chlorophylles naturelles* raised by Lubimenko will also have to be decided. Lubimenko's (28) observations of differences in leaf spectra on which this concept is primarily based are, unfortunately, all visual and made with a microspectral ocular of low dispersion with which the accurate determination of absorption maxima is difficult. While no careful spectrophotometric check of these reported differences has yet been undertaken, it appears probable from inspection of some of the data now at hand that these apparent discrepancies are due either to the varying thickness of the leaves studied, or, in some cases, to the shifting of the absorption spectrum by the intense illumination used. His second reason for believing in this multiplicity is the fact that he obtained

different yellow pigments from different plants. This has already been commented on above. His last reason relates to differences in the solubility in water and is well substantiated by the experimental evidence. It is, indeed, highly probable that considerable differences do exist in the albuminoid fraction of the pigment and that this is so is indicated by the ability of certain green algae to withstand temperatures up to 70°C. in hot springs. This point is to be investigated at an early date.

It remains only to list the remaining common cell pigments of the green and other plants. Very little work has been done on most of them in the living condition. From the green plant we obtain on extraction, in addition to chlorophyll, the unsaturated hydrocarbon pigment carotin, $C_{40}H_{56}$, and the closely related xanthophyll, $C_{40}H_{56}O_2$. From the discussion above it will be inferred that not much is known about their natural condition. It has been supposed by Wurmser (54), and others, that they are present chemically unchanged, in solution in a lipid of high index of refraction. Their function, even, is not definitely known, though a variety of rôles in respiration and photosynthesis have been assigned to them by various authors. The anthocyanins and anthoxanthones, derivatives, respectively, of benzopyrylium and benzopyrone, are apparently present in the cell sap in aqueous solution, usually as glucosides. Their function is not clear, but it appears to be related to the carbohydrate metabolism rather than to photosynthesis.

From the brown algae and diatoms we obtain on extraction, in addition to chlorophyll, carotin, and xanthophyll, a third carotinoid, fucoxanthin, $C_{40}H_{54}O_6$. This is present in an amount about equal to the sum of the other two carotinoids and is believed by Willstätter and Stoll (52) to be responsible for the olive coloration of these plants. There is, on the other hand, the entirely different view of Reinke (41), and of Molisch (31), that in the living alga or diatom we have, instead of phyllochlorin, a brown pigment, phaeophyll, which is rapidly changed into chlorophyll by heat or alcohol. This would account for the rapid color change from brown to green which takes place on heating. It is difficult to explain this change if the olive color is due to fucoxanthin, unless we assume that there is a considerable change in the fucoxanthin absorption spectrum on heating, as the color change is far too great to be due to the usual phyllochlorin-to-chlorophyll shift alone. An investigation of this question is contemplated. A still earlier explanation is that due to Millardet, who believed that the olive coloration was due to a brown water-soluble pigment, phycophaein. The work of Tswett (50) and of Kylin (23) has apparently shown that the brown pigment of aqueous extracts is an oxidation product which rapidly forms under the influence of the slightly alkaline reaction of the extract. It also is said by Kylin to be closely related to the tannins.

The red and blue-green algae give on extraction with fat solvents the

same pigments as do the green plants. In addition to these they also contain water-soluble pigments, which, according to Kylin (22) and others are proteins with a prosthetic chromatophore group. These pigments are fluorescent, and apparently photodynamically active in photosynthesis, and are divided into two groups. The red pigments, or phycoerythrins, are present in considerably greater quantity in the red algae than in the blue-greens, and all that have been described are apparently closely similar. The bluish pigments, or phycocyanins, vary more widely, notably in their spectral characteristics. Kylin (21, 22) has described three distinct varieties, blue-green, blue, and blue-violet phycocyanin, and recently Svedberg and Katsurai (47) have determined the molecular weights of the first and third of these under the names of *c*-phycocyan and *r*-phycocyan, respectively. Some spectrophotometry of the organisms containing these pigments has been done in connection with the study of "chromatic adaptation," as for instance by Gaidukov (9), but little is known about the pigments *in vivo*. A beginning has recently been made, using the photographic spectrophotometric technique, in the investigation of the thermostability of these pigments *in situ*, but the results are not yet ready for publication.

The only bacterial pigments which will be mentioned are those of the purple, brown, and green bacteria. The first two of these will be considered together, as recent unpublished work of Dr. C. B. van Niel has shown that under proper culture conditions brown bacteria come to resemble the purples in appearance, and preliminary spectrophotographic observations indicate that the brown color is perhaps only the result of a different admixture of the usual pigments of the purples.

The entire pigment complex of the living purple bacteria was given the name of bacteriopurpurin by Lankester (24) as long ago as 1873. Its interesting spectrum, including three bands in the near infra-red, has been studied by Engelmann's bacteriogram method by Buder (5), and Engelmann (8) himself made a spectrophotometric determination in 1888. It seems to be photodynamically active throughout its range of absorption. Recently Becking (2) has compared the absorption spectra of several different purple bacteria spectrophotographically, but no attempt has been made to determine the nature of the *in vivo* pigment or pigments. When the bacteria are extracted with absolute alcohol a bright green pigment, bacteriochlorin, is obtained. This pigment, first isolated by Nadson (32), differs spectroscopically and otherwise from chlorophyll. Subsequent extraction with chloroform, or carbon bisulphide, gives a solution of a bright red pigment, the bacterioerythrin of Arcichovskij (1). Practically nothing is known about the chemical nature of these pigments, although it has been suggested that bacterioerythrin is related to the carotinoids because of its absorption spectrum, solubilities, and blue color with H_2SO_4 .

Very little is known about bacterioviridin, the pigment of the green bacteria; in fact, not much is known about the bacteria themselves. Until recently there has been a difference of opinion as to whether these organisms were bacteria or minute chlorophyceae, and there is still doubt as to whether bacterioviridin is or is not chlorophyll. Perfiliev and Monteverde (38), in 1914, came to the conclusion that the two pigments were closely related on the basis of the similarity of the red fluorescence and absorption bands of their alcohol extracts, as well as reactions to acid and alkali. Metzner (30), in 1922, also examined the spectrum of the extracted pigment and concluded that it was not chlorophyll. One attempt has been made to determine the spectrum of the *in vivo* pigment of green bacteria cultured by Dr. van Niel, but it was not successful owing to the very large amount of sulphur with which the bacteria are ordinarily surrounded.

Enough has now been said to indicate what a wide and fertile field for research is to be found in the study of the pigments of the living cell by physico-chemical methods. The employment of the apparatus and methods of the physical laboratory in biochemical work is, of course, not a recent development. What is new is the realization that such methods as spectrophotometry, instead of being useful only for descriptive purposes, or to supplement other evidence, are, when properly applied, a source of primary data for which a chemical or physical interpretation must be sought.

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XV. CELL MEMBRANES AND CELL BRIDGES IN THE FORMATION OF BLASTULA AND GASTRULA

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THE BLASTULA

One of the major problems in biology is that of the unity of the organism. What are the means used to bind together cells and groups of cells into an interdependent community? In the larger forms of life, circulation and nervous tissue are special systems adapted to this end. But the early embryonic stages are not served by these systems, and yet we must grant that the blastula is an individual organism. The problem indeed presents itself with the first cleavage, for frequently, though not in all cases, the development or non-development of one blastomere profoundly affects the subsequent history of the other.

The simple protozoan differs from the metazoan in that for the simple protozoan growth is only a question of cell-division, but for the metazoan growth involves more: the cells which result from division must be held together. Considerations of the earliest phases of development have been largely centered in the mechanism of cell-division without regard to the problem of cohesion. Necessarily the two phenomena work in senses opposite to each other. A process which results in separation will hardly at the same time work to maintain adhesion. Therefore, while changes in surface tension may possibly explain the division of the mother-cell, it seems clear that another set of phenomena is necessary to account for the daughter-cells remaining in contact through subsequent cycles. As a matter of fact it can be demonstrated that this is true for the developing eggs of the sea urchin and the sand dollar, and that the twofold mechanism for this purpose is that of membranes and cell bridges. In the third place, following cell-division and the cohesion of the blastomeres, the latter must not only be held together but held together in such fashion as to form a blastula. In his work, *On Growth and Form*, D'Arcy Thompson (19) speaks of the two associated problems of blastula and gastrula formation as: "(1) the formation and enlargement of segmentation cavity, or central interspace around which the cells tend to group themselves in a single layer, and (2) the formation of the gastrula, that is to say, the conversion by invagination of the one-layered ball into the two-layered cup. . . . The former problem is comparatively easy, as regards the tendency of the segmentation cavity to enlarge *when once it has been established*." In this last phrase (*italics mine*) the problem of the formation of the blastula is passed over or assumed. We shall see, however, that there is no justifica-

tion for this assumption, that the formation of the blastula is not the inevitable consequence of segmentation but is a problem, quite as real as those of cell-division and cell-cohesion.

Let us then seek an answer to the question by the procedure of eliminating a structure in order to discover its function and study the development of eggs which have been deprived of their membranes (11, 13). This can be accomplished in two ways with the eggs of *Dendraster*: (1) formation of the fertilization membrane is prevented by keeping the unfertilized eggs for ten minutes in a solution of non-electrolyte, then fertilizing them in sea water; (2) freshly fertilized eggs are put into a solution of non-electrolyte until the fertilization membranes dissolve. In each case although there is no membrane the eggs divide regularly and give rise to blastomeres which cling together; and finally, as a result of cleavage, the cells are the size of those in normal blastulae. There is this difference, however, that the cells instead of forming a globe, form plates, more or less curled up at the edges (Fig. 1).¹ Membranes are therefore necessary to the

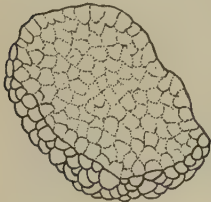


FIG. 1.—Cell-plate of *Dendraster*, advanced stage, showing cup-shaped form.

formation of the blastula. How do the membranes normally prevent the blastomeres from forming a plate? It is evident that in normal development, the third cleavage is the first step in the formation of the spherical embryo. But in eggs developing without membranes the third cleavage plane, in some or all of the blastomeres, is again vertical and not at right angles to the first and second planes. This necessarily results in a flat, plate-like structure, which in the later stages becomes curved at the edges. Driesch's experiment (7) in which he altered the cleavage planes of echinoderm eggs by means of pressure between plates sug-

gests that contact with the hard surface plus gravity may explain the alteration of the plane of the third cleavage, since eggs deprived of membranes necessarily lie in contact with the glass bottom. Normally, the dividing cell rests on a cushion of liquid within the fertilization membrane, which protects it from such action. We may suppose, then, that the membrane preserves the proper position of the cleavage planes by keeping the egg cushioned so that it never rests directly on a hard surface. It therefore becomes a matter of fundamental importance to determine the conditions essential to the formation and maintenance of the fertilization membrane.

It can be shown that this depends upon the presence of a bivalent metal ion, for if Mg, Ca, Sr, or Ba ions be added to the solution of non-electrolyte to which the unfertilized eggs are subjected, then upon fertilization true membranes form, provided the amount of salt is sufficient. Table I

¹ All figures except Figure 10 drawn from camera lucida sketches by Mr. Whong.

shows the amounts of the salts which must be added to 50 cc. of urea to protect the power of sea urchin eggs to form membranes when they are fertilized.

TABLE I

50 cc. M or M/ ₁ Urea Solution	Results of Fertilization
$\frac{1}{2}$ cc. MgCl_2	Hyaline membranes; only few blastulae.
1 cc. MgCl_2	Fertilization membranes; normal blastulae.
$\frac{1}{2}$ cc. CaCl_2	Hyaline membranes only.
1 cc. CaCl_2	All membranes normal; normal blastulae.
$\frac{1}{2}$ cc. SrCl_2	Hyaline membranes; no larvae.
1 cc. SrCl_2	All normal membranes; normal blastulae.
$\frac{1}{2}$ cc. BaCl_2	Hyaline membranes; no blastulae.
1 cc. BaCl_2	A few normal membranes; some normal blastulae. Most of the eggs killed.

It is evident that the four ions are equivalent in their protecting power. As a rule in physiological reactions 1 Ca ion is equivalent to about 10 Mg ions, while Sr and Ba are even stronger than Ca (12). Lucké and McCutcheon (10) have found Ca and Mg equipotential in their effects on the absorption of water by the unfertilized eggs of *Arbacia*. They assume, therefore, that the ions exert this effect by virtue of their valence.

Furthermore, it can be shown with the eggs of *Dendraster* that fertilization changes the ratio of effectiveness of the ions.

TABLE II

DEVELOPMENT OF UNFERTILIZED EGGS OF DENDRASTER

Added to each 50 cc. M or M/ ₁ Urea	$\frac{1}{4}$ cc.	$\frac{1}{2}$ cc.
MgCl_2	No	15% blastulae
CaCl_2	develop- ment	50% blastulae
SrCl_2		20% blastulae
BaCl_2		20% blastulae

This shows the approximate equivalence of the four bivalent metal ions with the possible exception of Ca, which gives a higher percentage of development than the others when $\frac{1}{2}$ cc. is used.

TABLE III

MEMBRANE PROTECTION IN UNFERTILIZED EGGS

MgCl_2	CaCl_2	SrCl_2	BaCl_2
$\frac{1}{4}$ cc.	No membrane	25% membranes	None
$\frac{1}{2}$ cc.	50% membranes	75% membranes	75% membranes
1 cc.	100% membranes		
4 cc. 75% membranes			

The percentages indicate the extent of protection of the membranes previously formed in fertilization. The figures show that, while the ions Ca, Sr, and Ba are practically equivalent in their protective action, Mg has about one-eighth their effectiveness. This is the usual physiological relation between Mg and Ca. The fact that the ratio of Mg to the other bivalent ions approximates unity for protecting the mechanism of membrane formation in the unfertilized egg and is nearly eight times as great after the fertilization membrane is formed suggests that we are dealing with chemically different systems in the two cases.

A word should be said here about the fertilization membrane as to whether it is formed at fertilization or already exists on the unfertilized egg. Micro-dissection shows that the unfertilized egg of *Dendraster* is enveloped with a fragile membrane and that this dissolves off in the solution of urea. Much has been made of the existence of a membrane on the unfertilized egg of the starfish and the sand dollar, some going even so far as to contend that this fragile membrane is the fertilization membrane, and that at fertilization it is simply lifted from the egg (see *Heilbrunn* 8). It should be noted that the membrane of the unfertilized egg is extremely fragile, easily pierced or torn by the needle, while the fertilization membrane is tough and resistant. There is also the fact that Mg protects the membrane of the unfertilized egg as well as do the other ions but is only one-eighth as effective in protecting the membrane after fertilization. A fair statement then would be that the fertilization membrane has not, before fertilization, attained either its physical or chemical form. All one can say is that the membrane surrounding the unfertilized egg may act as a nucleus upon which the fertilization membrane is formed. However, since fertilization and segmentation take place without any membranes at all, the whole question of whether the membrane is formed or "elevated" is one of entirely minor importance. What is of prime importance, however, is the part the membrane plays in the conversion of a group of segmenting blastomeres into an individual blastula. We have shown that the presence of a membrane is essential to this transformation. In order correctly to evaluate the rôle of membranes in this process, we must first inquire into the occurrence and function of the cell-bridges. These are of two kinds, the primary bridges formed as a direct result of division, and secondary bridges which form when two embryonic cells remain in contact a short time.

A study of the first division in eggs without membranes clearly shows a hyaline bridge connecting the two blastomeres, the primary cell-bridge (Fig. 2, *b*, *d*). Plough (15) in separating the blastomeres has noted such a structure and Conklin (6) says, "with all eggs with which I am acquainted connections do persist for a considerable period, at least at the point where the cells have last separated, that is, at the point of the mid-

body." This bridge is elastic as shown by the fact that if the two cells are pulled apart by means of micro-dissection needles and then released, they quickly return to their former position near each other as if connected by an elastic band. When the second division takes place, two new bridges

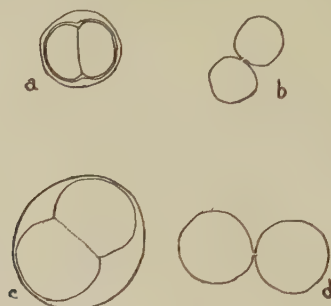


FIG. 2.—First division of cell; a, normal 2-cell stage of *S. purpuratus*; b, same without membrane; c, normal 2-cell stage of *Dendraster*; d, same without membrane.

are formed. Each is seemingly the result of the failure of the cells to pinch entirely in two. This results in a group or, with shaking, in a chain of four cells (Fig. 3), which, if numbered 1, 2, 3, 4, are bridged in this

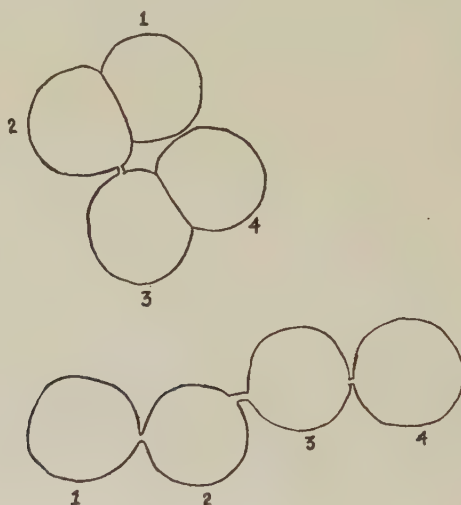


FIG. 3.—Four-cell stage of *Dendraster*, without membrane, showing primary cell-bridges between blastomeres 1 and 2, 2 and 3, 3 and 4, but none between 1 and 4.

wise: 2 and 3 are joined by the original bridge, 1 and 2, 3 and 4 by the bridges resulting from the second division. But 1 and 4 are never connected by a primary cell-bridge. In the 16-cell stage the position of the

micromeres shows that 1 and 4 represent the animal pole, 2 and 3 the vegetal pole.

In the older literature suggestions were made from time to time that the cells of any given organism are connected in some way. In 1893 E. B. Wilson (20) expressed the view that the unity of the metazoan organism demanded protoplasmic connections between the cells. He says: "These facts as well as those afforded by double embryos show that the unity of the normal embryo is not caused by a mere juxtaposition of the cells. They indicate that this unity is not mechanical but physiological and point toward the conclusion that there must be a structural continuity from cell to cell that is the medium of co-ordination and that is broken by mechanical displacement of the blastomeres." This view regards the organism as a syncytium and coincides with the theory of Sedgwick (17) as to the structure of the nervous system.

Over thirty years ago, G. F. Andrews (2) and E. A. Andrews (1) described, as a result of extensive studies using the highest magnification of the compound microscope, the existence of "filose processes," which are formed by the fertilized egg and blastomeres. These processes they likened to the protoplasmic threads of the Heliozoa, and ascribed to them a twofold function in the embryo, namely, (1) contraction, by means of which the blastomeres are held together, (2) paths of conduction, by which the cells of an organism exchange material. Their view was substantially identical to that of Wilson and of Sedgwick. Mrs. Andrews says in *The Living Substance as Such and as Organism* (p. 87): "... it becomes evident that so long as there is between the units composing it a protoplasmic connection, even the most delicate, a whole colony must be conceded the value of a morphological unit. For there is no true difference between a connection such as is shown in Vorticellidae, where the contractile stalk of each unit is continuous with all the others by way of the common stem, and that seen to be established in a compound Heliozoan or compound Coelenterate, or even in a developing starfish egg."

Later workers were less successful in establishing the existence of "filose processes" or cell-bridges, and Chambers (5), on what must now be considered insufficient grounds, shows himself skeptical of their existence. Wilson in the last edition of *The Cell* little more than mentions them. Yet it must be said that if cell-bridges exist and function, then they are structures of primary importance in the construction of the metazoan body.

It is evident that the slight consideration accorded these structures in the past thirty years is due to the difficulty of demonstrating them. The chief obstacles to separating the blastomeres, and by this means laying bare the hyaline filaments binding them together, are the fertilization and hyaline membranes. The problem then is to get rid of these masking structures, and this can be done by means of ion-free solutions.

However, it is evident that the primary cell-bridges which are so easily seen after the first and second cleavages are insufficient to form the tightly knit blastula, or the cell-plates corresponding to the blastula. Now with the progress of division the cells of a plate become more closely knit. In the 8- and 16-cell stages the blastomeres are loosely clustered, with great spaces between some cells of the cluster (Fig. 4). With further cell-

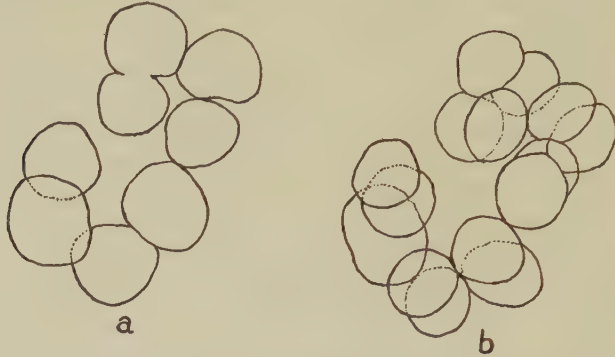


FIG. 4.—*Dendraster*, *a*, showing 8-cell stage of larva without membrane; *b*, showing 16-cell stage. Note open jaw form, the opening being the animal pole.

division the intercellular spaces are first reduced in size and then finally obliterated altogether (13). Another curious phenomenon may be observed in the cell-plates, for as the cells pass through the various phases of nuclear and cytoplasmic division at one time they round up and later they flatten against each other. These phases in summation cause a striking effect. At one moment the plate may be fairly flat, but in a few minutes it may be rounded up at the edges and occupy considerably less area in projection than before (Fig. 5). The plate shows an effect which may aptly be termed systole and diastole. This is evidence in itself that the cells are closely joined. In fact, such is the case, and it can be demonstrated that numerous hyaline bridges bind adjacent cells together. In the first place, if a plate be shaken apart it will be seen that any two cells may be joined by several bridges and not by a single one, for in the plate fragments two or three or even more hyaline processes extend out from the cells on the edge. The fact that each cell may show several bridges proves that these are not a result of cell-division but are formed *de novo* between adjacent cells, and are therefore secondary cell-bridges. Direct evidence of the power of the cells to form cell-bridges at any time is furnished by the following experiment carried out by my assistant, Mr. S. H. Whong. By means of micro-dissection needles he brought cell-groups from two different eggs together and held them in contact for from fifteen to twenty minutes. At the end of that time he found that the cells in contact had

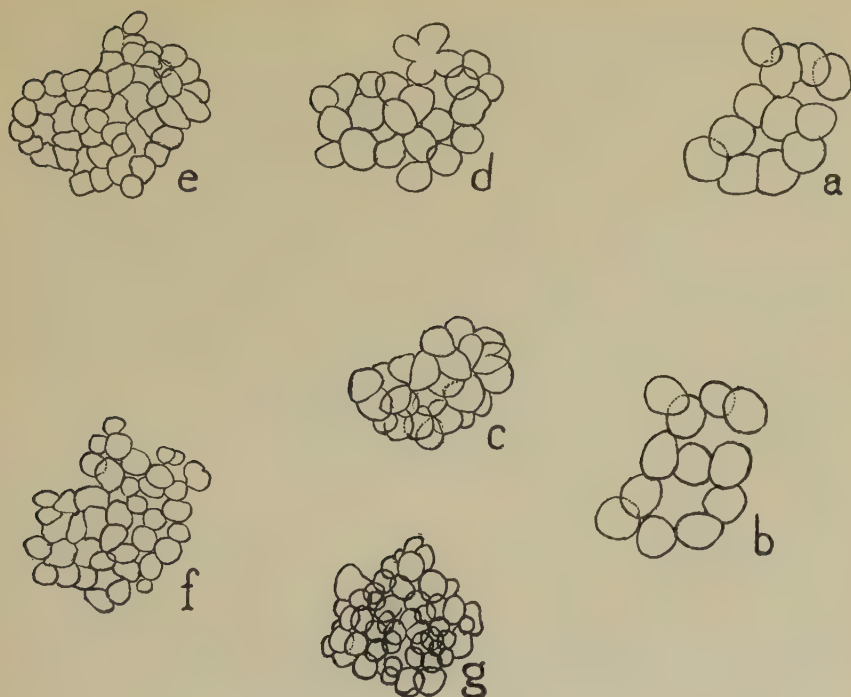


FIG. 5.—The same cell-plate at different times figured to show the phases of contraction and relaxation: *a*, at 1:20 o'clock; *b*, at 1:25; *c*, at 1:50; *d*, at 2:10; *e*, at 2:20; *f*, at 2:25; *g*, at 3:00 o'clock.

become joined along the line of contact. By pulling them somewhat apart the new cell-bridges were seen. This experiment succeeded in the case of the 2-, 4-, 8-, 16-, and many-cell stages. It is clear, then, that secondary cell-bridges begin to form as early as the close of the first division, and the 4-cell stage shows many of them.

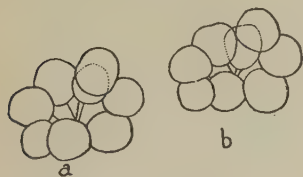


FIG. 6.—Contractile action of the secondary cell-bridges. In *a*, after division the blastomeres lie far apart; in *b*, just before division they are drawn together by contraction of the secondary cell-bridges.

The secondary bridges form only as a result of contact between the blastomeres and, as the figures show, are much finer than the primary bridges. Their action is shown in Figure 6, *a* and *b*, in which it can be seen that shortly before cell-division the bridges by contracting draw the blastomeres together. Just after each cell-division the bridges seem to become extensible and lose for the time their contractility, as shown by the appearance and enlargement of openings between the cells. Later extensibility seems to cease and after the 128-

cell stage the cells are permanently held together. It seems possible then that cell movements, the causes of which have often been referred to viscosity and surface tension of the blastomeres, are really due, at least in part, to the rhythms of contractility and extensibility of the cell-bridges.

It should be specifically stated that in our experience functional cell-bridges arise only as the result of contact between the blastomeres. They are never spun across an open space as stated by E. A. and G. F. Andrews. The only conclusion to be drawn is that the structures which they described as being thrown out into the surrounding fluid space were something else. Furthermore, the secondary cell-bridges are of fine structure and scarcely effective in the earlier stages, because of the relatively great mass of the blastomeres, but later as the mass of each cell becomes smaller the secondary bridges come to be of increasing importance.

In sum, then, the observations prove that there are three types of structure necessary to the formation of the blastula: (1) the membrane or membranes resulting from fertilization; (2) the primary cell-bridges; and (3) the secondary cell-bridges. The membranes (*a*) keep the blastomeres from contact with a hard surface, thereby maintaining the condition necessary for the third cleavage plane to fall horizontally; (*b*) prevent the blastomeres in the 4-cell stage being disturbed and shaken apart into a chain. Both these functions are necessary to the first steps in the formation of the embryonic sphere. The primary cell-bridges keep the blastomeres from falling apart, within the membrane, where no hyaline membrane is present, and by their contractility at all times keep the cells in close contact. This last function is, in our experience, necessary to the formation of the secondary cell-bridges. Without direct contact and subsequent formation of the secondary bridges, cells not connected with each other by primary cell-bridges would tend to fall apart. Thus every early embryo would show a great chasm to the extent of a half-equator passing through the animal pole, since there are no primary bridges between blastomeres 1 and 4 and their abutting derivatives. Since normally the membrane holds 1 and 4 in contact, they are soon united by secondary bridges, and the resulting sphere is closed.

A question closely related to the foregoing facts is that of the cause of the formation of identical twins, i.e., two individuals from the same egg. The early studies of Loeb (9) on this subject led him to the conclusion that the formation of identical twins due to the failure of the first two blastomeres to coalesce was the result of, first, a watery layer being thrust between the two cells; and, second, the solution and disappearance of the hyaline membrane, thus allowing the two blastomeres to drift apart, so that they developed out of contact with each other. In each case, according to Loeb's view, twin formation depends upon complete separation of

the blastomeres and their subsequent independent development into two larvae of half-normal size. Such a view could not account for partial or "Siamese" twins.

It seemed possible that the development of the eggs of *Dendraster* might furnish further information on the phenomenon of twin formation, for the reason that in this egg there is no functional hyaline membrane binding the blastomeres together, and the fertilization membrane is not of sufficient diameter to allow the blastomeres to lose contact with each other (Fig. 7). And yet the formation of twins and even of quadruplets



FIG. 7

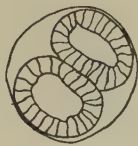


FIG. 8a

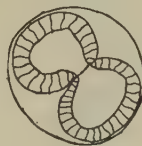


FIG. 8b



FIG. 9

FIGS. 7-9.—Various stages of development of *Dendraster*: FIG. 7.—Two-cell stage of *Dendraster* kept in a solution made up of 100 cc. NaCl m/2, 2.2 cc. KCl m/2. When put into sea water such an egg develops into twins of half-size. FIG. 8.—Twin formation in *Dendraster*, blastula stage: a, completely separated twins; b, incomplete or "Siamese" twins. FIG. 9.—Quadruplets formed by removing the larvae from the artificial solution to sea water in the 4-cell stage.

takes place when the fertilized eggs are kept in the following solutions: 5 cc. $MgCl_2$ + 50 cc. NaCl, or 1.1 KCl + 5 cc. $MgCl_2$ + 50 cc. NaCl, the solution being isosmotic with sea water. In these solutions divisions of nucleus and cytoplasm take place after the usual time intervals, but the blastomeres remain rounded and do not flatten against each other. If these eggs are returned to sea water after the first division, subsequent division goes on normally, but the plane of the first cleavage will not be spanned by cell-bridges for lack of contact; consequently the blastomeres develop as separate organisms (Fig. 8).

For the same reason, if the eggs are left in the artificial solution until after the second cleavage, quadruplets form (Fig. 9). Beyond this point the experiment cannot be carried, even though the blastomeres of the 16-cell stage are equipotential with regard to blastula formation. The clue to this failure seemed to lie in the amount of free outside surface which each blastomere possesses. For it can be seen in the artificial solution, which lacks Ca ion, that cell-division goes on apparently normally, but the cells remain rounded even in advanced stages. If such larvae are put back into sea water at any time after the 8-cell stage they develop into normal-sized blastulae. A consideration of the positions of the blastomeres with reference to each other suggests that the important variable and the

immediate cause of twin formation is the extent of free outside surface possessed by each blastomere. By free surface is meant surface which does not come into contact with that of another blastomere. To make the point clear let us consider spheres arranged in a circle in one plane (Fig. 10). In the case of the blastomeres the points of contact soon become faces

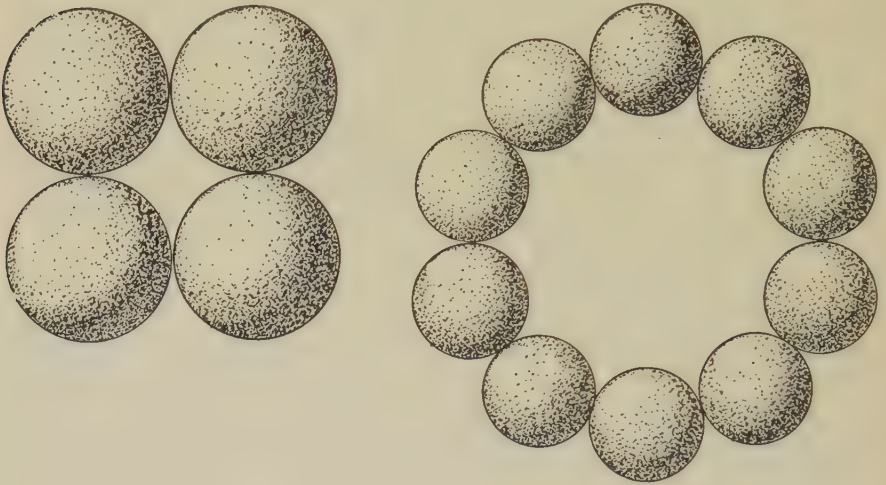


FIG. 10.—Diagram showing ratios of inside to outside surface as defined by the points of contact. It is apparent that as the number of spheres increases this function approaches unity as a limit.

of contact, owing to the lack of rigidity in protoplasm. This tendency to flatten will be greatest from the point of contact toward the central space or blastocoele, since the greatest pressure is exerted here. Consequently the free surface of greatest extent will be that on the outside. Now it is evident that, as the number of spheres in the circle increases, the ratio of surface inside the cavity to that on the outside approaches unity as a limiting value. It seems therefore that this ratio may be of significance for the formation of twins. Now it can be seen from geometrical figures that if spheres grouped in contact be considered in reference to the relation of their free outside surface to that inside as delimited by an imaginary sphere passing through the points of contact, a certain relation results. The function is a simple one in which the ratio $R = \frac{1 - \sin \pi/n}{1 + \sin \pi/n}$, where n equals the number of spheres. The following table of values is suggestive.

TABLE IV
TABLE OF VALUES

<i>n</i>	<i>R</i>	<i>n</i>	<i>R</i>
2	0	32	.82
4	.17	50	.88
8	.45	100	.94
16	.67	1000	.99

It is of course apparent that only for the first two values, i.e., the first and second cleavages, does the actual situation of the blastomeres correspond to the formula, i.e., all the spheres in one plane. But the higher numbers represent an approximation to the truth. The fact that the function increases very rapidly from the first applies to the cells of a morula either in one plane or in many. Experience shows that as the value of *R* becomes sensibly great, i.e., $> .2$, the amount of free surface outside is diminished to such a point that independent development of the blastomere becomes impossible. The fact that the spheres which are the blastomeres are not rigid but plastic, results, as we have said, in the points of contact becoming planes of contact. This makes possible the union of the cells by secondary cell-bridges. The more extensive this process the more surely is the larva a unit, and the less the possibility of the blastomeres developing independently into multiple blastulae. The conditions for twin and quadruple formation are then: minimum contact between the blastomeres; maximum outside free surface; and, secondarily, as a result of the first condition, minimum formation of cell-bridges.

It is clear that the smaller the number of spheres the more favorable is the first condition. But such a condition must be maintained for some time if multiple embryos are to result. For this the rhythms of viscosity and surface tension are of first importance. High surface tension and low viscosity are responsible for rounded form. In these experiments, permanence is given to the free surface of the rounded blastomere by some chemical change which is the result of the ionic unbalance of the surrounding solution.

THE GASTRULA

Apart from the neo-vitalistic view of Driesch, there are three theories to account for gastrulation. Rhumbler (16) showed by means of a mechanical model that if the cells at the vegetal pole of the blastula actively change their shape so that the broad base of the cell is inward, then the result must be an invagination. He supposes this to be the mechanism of invagination of the gastrula. This theory throws upon the fluid cell the

work of changing its shape against counteracting forces and maintaining this form with a certain measure of rigidity. The objection was early made that isolated cells of the amphibian blastula quickly become globular. I was able to show the same effect with the cells of the evaginating gut of *Dendraster* larvae. By means of a micro-dissection needle a larva was brought to the air-water surface of a hanging drop, with the vegetal pole in contact with the surface. At once the larva was caught by the surface film and seemed to explode. The endodermal cells were scattered like dust in the drop. These cells were seen to round into spheres, and there was no evidence that they ever tended to assume any other than a globular shape. Nor did they show any force of attraction for each other. Furthermore, it should be noted that even *in situ* the cells surrounded and in contact with other cells are constantly changing their fluidity, their viscosity, and their form. One cannot suppose, then, as Rhumbler has, that endodermal cells can assume shapes of comparative rigidity and thereby confer upon the embryo a new structural form.

Bütschli's (4) suggestion of differential water absorption by the blastula wall is no more plausible in the light of experimental evidence. Spek (18) has applied Bütschli's theory to account for the evagination of sea-urchin larvae in lithium sea water. He assumes that lithium causes the outer layer of the blastula wall to become lyophilic as compared to the inner wall. A consideration of one of Herbst's lithium experiments proves such an explanation inadequate. If developing eggs of sea urchin or sand dollar are kept in 50 cc. sea water plus 2 cc. LiCl up to the 300-cell stage and are then transferred to sea water, they develop into exogastrulae. On the other hand, if normal larvae in the 300-cell stage are put into lithium sea water and kept in it until gastrulation, they invaginate normally. Lithium then must be applied very early, before the 300-cell stage, in order to give the necessary bias to the blastomeres so that evagination will take place. In fact, if the Bütschli-Spek hypothesis were correct, then the larvae which were started in lithium sea water and later developed and evaginated in pure sea water should invaginate more strongly than in the normal, since all of the Li will be in the blastocoele. But this is just the opposite of what happens. A simple colloidal explanation of gastrulation therefore fails.

In 1910 Assheton (3) made a suggestion as to the nature of the process of invagination, to the effect that if the cells of the blastula attract each other, and that if the centers of attraction are eccentric, then any increase in pressure will result in a buckling, and this will be "in" if in a certain region the centers of attraction between the cells are disposed toward the periphery. Assheton's study of the geometrical relations between nucleus and cell in the blastula of *Amphioxus* showed that in all cases the nuclei

at the vegetal pole are nearer the periphery than at the sides and the animal pole. Therefore he suggests that the nuclei may be taken as indicating the centers of attraction between the cells.

A study of sections of echinoderm blastulae shows that the nuclei are placed just as in Assheton's sections of blastulae of *Amphioxus*, namely, at the animal pole the nuclei are disposed toward the centers or even the inner surfaces of the cells, while at the vegetal pole they occur eccentrically placed toward the outside.

It seemed to me that the crucial test of Assheton's theory would be an experimental one. In lithium larvae, evagination takes place, and in that case, if Assheton's view were true, the positions of the nuclei should be reversed and the nuclei at the vegetal pole should lie next the blastocoele. The event showed that in lithium larvae *all the nuclei* were disposed toward the *outside*. Further experiments showed this displacement of the nuclei to be an ion effect independent of whether invagination or evagination took place. Hence, it must be concluded that the nuclei do not form centers of attraction between the cells (14).

The central idea of Assheton, however, remains good, and the possibility exists that there may be material bonds of attraction between the cells. The cell-bridges which we have described may very well carry out this function. It need only be supposed that they can be asymmetrically placed

and can exert contractile force, to account sufficiently for either invagination or evagination.

Evidence that cell-bridges join cells asymmetrically is given by plate fragments (14). These yield cell chains which are bent (Fig. 11). The only way in which bending could occur would be in the asymmetric attachment of the cells, and these attachments, as we have shown, are cell-bridges.



FIG. 11.—Groups of cells of cell-plate of *Dendraster* detached, showing bending in the chain caused by asymmetrical attachment of the cells.

If we are to take the view that gastrulation is the result of cell-bridges developing eccentrically and forming bonds of attraction between the cells of the endoderm, we shall have to admit that anatomical specialization in the development of organs begins very early. It is pertinent to this question to refer to the fact that an evaginating gut in *Dendraster* very early grows at an angle to the face of the larva from which it springs, which angle is the mirror image of the one formed in the normal larva in order to approximate the stomodaeum.

In conclusion it should be said that the view of the structural unity of the early embryo set forth in the preceding pages makes imperative a revision of our concept of the organism as a whole. Changes in surface

tension and viscosity of the cells may suffice to explain cell-division, but they are inadequate to account for the orderly arrangement of the cells in the organism. Definite structures, the secondary cell-bridges, are necessary for this; without these structures the blastomeres cannot form the embryo. While the cells are the substance of the organism, the connecting bonds make possible the orderly arrangement of the cells into organs, and into the organism of definite form. The animal body, then, is not merely an aggregation of microscopic units—the cells—but a syncytium, a morphological unit, the physiological consequences of which remain to be explored.

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XVI. THE EARLY DEVELOPMENT OF ORGAN ANLAGEN IN AMPHIBIANS

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INTRODUCTION

In this paper we shall deal especially with the development of hypophysis and thyroid anlagen of the California red-leg frog, *Rana aurora*, transplanted into a heterotopic position in older tadpoles than those from which they were taken. The work was supported by a research grant from the University of California.

The transplantation of organ anlagen is not new in principle, as witness the many experiments of Spemann (18), Harrison (8), Lewis (13, 14), Braus (6), Streeter (19, 20, 21), Detwiler (7), and a host of others too numerous to mention. Among the most familiar of these experiments are those that involve the transplantation of the limb-buds of frogs and salamanders to normal limb locations or to abnormal positions upon the head, back, etc. It has long been known that, while these transplants grow best when placed in normal (orthotopic) position, they will nevertheless grow after a fashion in abnormal (heterotopic) positions showing characteristic structures, thus clearly indicating that they contain inherent tendencies causing them to undergo "self-differentiation" in wholly abnormal surroundings. In the salamanders, the fore-limb buds have been chiefly used and in the anurans the hind-limb buds—these being in each case the first to appear. In most experiments these limb buds were removed at the stage when they constituted slight button-like elevations upon the surface of the body. Microscopic study reveals in them a thin outer layer of ectoderm inclosing a core of loose mesoderm cells without any suggestion of the bones and muscles that will later develop within. Very clever experiments of Harrison (9) have shown that the potentialities that shape the development of the limb reside not in the ectoderm but in the mesoderm, as shown by the transplantation of each separately. The developmental tendency is at first of a very general character, as shown by the fact that a single limb bud often forms two limbs either spontaneously when transplanted into an abnormal position, or when split in any arbitrarily chosen plane parallel with its axis. Two superimposed limbs will often develop as one. A right limb will develop as a left limb if it is transplanted in an inverted position that is rotated 180° with its ventral side directed dorsally (10).

It has recently been shown by Swett (22, 23), Brandt (5), and Detwiler (7) that the order in which the three dimensions of the salamander

limb become definitely fixed and incapable of reversal are, first, the antero-posterior dimensions; second, the dorso-ventral; and, third, the medio-lateral. There is thus a tendency, at first vague and later more sharply localized and differentiated. This is demonstrated in another way by the capacity of surrounding tissues to close in and regenerate the missing limb after the removal of the region of the limb bud or other organ anlage. This is marked in early stages but diminishes later as the developmental tendency becomes more sharply defined and more highly specialized. The same thing is shown by experiments in splitting anlagen in early and later stages.

These organ anlagen have been traced to very early stages that are being accurately investigated by experimental means. Detwiler (7) has recently excised the fore-limb anlage of *Ambystoma* at the very early stage in which the first faint lines of pigmentation foreshadow the appearance of the neural folds. These limb-bud anlagen did not show the slightest elevation or other visible differentiation, and it was necessary to infer their location; but when excised and transplanted to other portions of the body of a slightly older larva they developed into fore-limbs in a perfectly normal fashion, and at this very early stage they had shown polarization in the antero-posterior axis of the body.

Similar experiments have shown that the anlagen of the optic vesicles that form the retina and a portion of the iris are laid down when the neural folds are first clearly defined, and the same is true of the anlagen of the auditory vesicles that produce the membranous labyrinth of the ear. We might multiply examples to show that the establishment of early anlagen capable of self-differentiation is a quite general principle of development. To date, these are the earliest stages to which the organs discussed above have been traced, but Spemann (18) and Mangold (15, 16) have shown that in the very early gastrulation stage one can exchange a patch of prospective neural plate with a patch of prospective surface ectoderm and each will develop according to its new position and not according to the source from which it is derived; in other words, these parts of the body have not yet been stamped with a definite developmental tendency. At the same time, Spemann, Mangold, and their associates have cleverly given us some knowledge of the mechanism by which these developmental tendencies will be established. They have shown that transplantation of the dorsal lip of the blastopore to a position under the ectoderm of the ventral part of the body will induce the formation of a neural tube from prospective integumentary ectoderm.

Time forbids our discussion of the brilliant work of this group of investigators upon the rôle of the organizer in development, and this is not necessary because the subject was so ably discussed by Professor Brachet at our meetings last year. I need only remind you that in the

eggs of many frogs the occurrence of the grey crescent upon the surface of the egg before the first division plane has appeared marks out the symmetry of the future embryo so that it is possible in the one-cell stage to determine the cytoplasmic regions that will normally enter into the formation of different portions of the embryo that will develop from the fertilized egg. Professor Brachet (4) himself showed that this organization of the unsegmented egg, while determined by the point of entrance of the sperm, is not determined by the point of puncture when stimulus to development is given by pricking with a needle.

All of this has led us rather far afield, but we may sum up our knowledge of the basic principles that determine the origin and development of organ anlagen by saying that they are at first general in character and vaguely localized and that with the progress of development they become more intricate in constitution, more sharply localized, and less susceptible of change. From very early stages long before they show visible structural features, they have definite potentialities and possess the capacity for self-differentiation.

EXPERIMENTS

With these principles as a background we shall discuss some recent original experiments in the transplantation of the anlagen of the hypophysis and thyroid gland together with adjacent tissues that involve principles similar to those discussed above. The work was performed upon *Rana aurora*, the anlagen being removed at their first definite appearance and placed in all cases into much older tadpoles of from 15 to 17 mm. total length. All of these transplants were heterotopic, being made into a pocket under the skin of the top of the head between the eye and otic vesicle. The first transplant was made on the right side of the head, and if a second was made it was placed upon the left side. It was thus possible to locate the transplants several months later in order to study them histologically. The transplant is merely thrust deep under the skin, no attempt being made to close the opening, which rapidly heals over. The operation is facilitated by the fact that vascularization takes place very quickly in this region near the anterior cardinal vein.

HYPOPHYSIS TRANSPLANTS

The anlage of the hypophysis was removed at the tail-bud stage and included a rather generous assortment of neighboring tissues, such as oral epithelium, pharynx roof, diencephalon, and mesenchyme, which gave variety to the results but made the study of the hypophysis transplant itself rather difficult. The growth of this transplanted embryonic tissue caused the formation of a conspicuous tumor-like growth that protruded from the top of the head like a horn. In cases where there were

right and left transplants the effect was rather bizarre. The buccal anlage of the hypophysis which gives rise to the pars anterior, pars intermedia, and pars tuberalis of the hypophysis develops as a narrow plate of cells growing from the surface ectoderm, where the mouth will later form, and extending caudally between the floor of the diencephalon and the roof of the pharynx. Although the transplant material was removed long before the mouth depression was evident and still longer before the appearance of the cuticular teeth, the included bit of prospective oral epithelium produced rows of cuticular teeth of characteristic form and arrangement. In some instances they were arranged above and below a well-defined mouth opening, all lying beneath the unchanged skin of the top of the head. The small quantities of loose mesenchyme that accompanied the transplant produced cartilage masses. In one case reconstructed by one of my students, Miss Viola Young, the portion of the cartilage adjoining the cuticular teeth was divided into two slender bars to which the teeth were attached, while the other end was more massive. There were no joints between these cartilage structures and the main mass, yet the whole configuration suggested that the teeth had at least modified the development of the adjacent cartilage.

Portions of the diencephalon have proceeded to form the characteristic brain structures easily recognizable. The study of the hypophysis itself was somewhat disappointing because of the distortion caused by the development of the neighboring structures discussed above. The characteristic eosinophile and basophile cells of the pars anterior were found, but it was difficult to estimate their quantity. While the experiment was designed to give a knowledge of the transplanted hypophysis and its functions, the work of last season upon this gland is chiefly valuable because of the by-products, such as teeth, cartilage, and brain elements. The work will be repeated the coming season to determine the development of the hypophysis anlage when carefully freed from adherent structures. We shall be interested to see how far it will be able to differentiate into its four component parts in the unusual environment into which it will be placed.

THYROID TRANSPLANTS

The thyroid anlage was removed at the stage when the external gills start to bud out, the total length of the tadpole being from 6 to 6.5 mm. A greater or less amount of mesenchyme was included with it, and this gave results of decided interest, as we shall see below.

In the majority of transplants where the anlage was not seriously injured, it developed into a thyroid gland of normal histological character. In this abnormal position under the skin of the top of the head, normal follicles were developed and they contained the usual quantities of colloid, showing the same reaction to Mallory's stain as that shown by

the thyroid gland of the animal into which the transplant was made. In some of the more successful cases there was no difference between the follicles of the thyroid gland of the host and those of the transplant. We can say with certainty that the heterotopic transplant develops into a normally functioning gland when placed under the skin at the top of the head. There were some transplants which showed one or more follicles filled with an abnormal fluid secretion that caused great distension; but other thyroid transplants were normal in every respect. It is quite probable that these cases of abnormality were due to injury inflicted in the operation.

In certain transplants little or no cartilage developed from the accompanying mesenchyme, while in others there were plates, rods, etc. In one very illuminating case the basi-hyoid cartilage developed with its characteristic keel. In normal development the thyroid anlage in the amphibians is closely applied to the edge of the keel and divides in two upon it, the halves migrating later to their more lateral position between the anterior and posterior cornua. In this single transplant in which a hyoid keel was formed, the thyroid anlage divided upon it in the normal way into right and left glands. This was true in spite of the fact that the hyoid cartilage chanced to lie in an inverted position and in a wholly unusual location. In another case where a cartilage rod was formed in association with the thyroid anlage the latter wrapped itself about in such a way as to envelop it but did not divide in two, although the amount of glandular substance developed was much greater than in the case in which the thyroid anlage was split in two.

In the other thyroid transplants there was no evidence of division of the thyroid anlage, and this corresponded with the absence of cartilage in the transplant or with the fact that if present, the thyroid anlage at least did not develop in proximity to it. This was an answer to the question that had arisen in my mind before undertaking the experiment, showing clearly that the thyroid anlage will divide in two only when it can come in contact with a cartilaginous hyoid keel. It is in line with my earlier observations in which an unpaired thyroid gland was sometimes observed to result from attempted thyroidectomies in cases where the anlage had been displaced but not removed.

EFFECTS OF TRANSPLANTS UPON DEVELOPMENT

It was demonstrated by Gudernatsch and by others that have succeeded him that feeding thyroid preparations to tadpoles induces precocious metamorphosis and it was independently shown by Allen (1) and Hoskins (11, 12) that absence of the thyroid gland results in their failure to metamorphose. Allen (1) and Smith (17), working independently, likewise showed that removal of the hypophysis prevents metamorphosis. Allen (2,

3) has shown that this process reaches the same stage of development in both thyroidectomized and hypophysectomized tadpoles, as well as in tadpoles from which both glands have been removed, the stage of hind-limb development in the absence of these glands differing in different species. It has been very fully demonstrated that both glands co-operate in producing the essential thyroid secretion, which is stored up in the thyroid gland alone.

It occurred to the writer that the transplantation of extra thyroid and hypophysis anlagen might result in the development of an abnormal sum total of glandular tissue and thus produce an unusual amount of effective secretion. In certain cases two extra thyroid anlagen were transplanted into a normal tadpole which would thus give it three times the normal amount of embryonic thyroid tissue. The same was done in the case of hypophysis transplants, and of course a single transplant as made in the majority of cases would result in a double amount of anlage material. In other cases one additional thyroid and one additional hypophysis anlage were transplanted with the hope of maintaining a balance in these glands.

Before we can announce positive results upon this line of experimentation, the work must be repeated upon a much larger scale and with the lessons of last season in mind. With 12 single thyroid transplants, 3 double thyroid transplants, 6 single hypophysis transplants, 5 double hypophysis transplants, and 3 with one thyroid and one hypophysis transplant, there is no evidence in any case of acceleration in metamorphosis, although the transplants of the hypophysis produced in two instances a marked darkening of the skin and in two or three cases a slightly less convincing darkening that would indicate increased secretion from pars intermedia substance of the hypophysis. These negative results upon the functional value of multiple hypophysis and thyroid anlagen are merely suggestive and must be repeated upon a large scale before we can draw any definite conclusions upon this fundamental problem. It would seem from the present status of the work that the functional significance of multiple embryonic anlagen is dependent upon the needs of the organism rather than upon the amount of embryonic tissue present.

Pasteboard models were constructed to represent the transplanted and the host thyroid glands. These were then weighed to determine the relative bulk, the term "weight units" being used to designate the weight of the models in grams. When we study the implanted thyroid glands and those of the host in order to determine the total bulk of secreting thyroid tissue, we find that in two—one a single thyroid transplant and the other a double one—the total amount of thyroid tissue is in excess of that found in the thyroid glands of normal controls. This is notably true in the single transplant, No. 30, in which the transplanted anlage has grown into

a gland with a value of 337.2 weight units as compared with 395 weight units that represent the sum of the weight of the pair of thyroid glands of the host tadpole. In the double transplant, the sum of the weight of the two thyroid transplants was 226.9 weight units and that of the host's own thyroid glands 356.9 weight units. It must be remembered that the latter figure represents the product of one thyroid anlage, while the former indicates the weight of two anlagen. It is evident that in neither of these two animals does the transplanted thyroid tissue equal that of the host, but in both the sum total is in excess of the normal, as shown by the control No. 62 with 505.9 weight units, which is in a comparable stage of development.

This material is, however, too scanty to give us a decisive answer to the question of the quantity of glandular tissue produced or to the very interesting question as to whether the transplanted thyroid gland inhibits the growth of the host thyroid glands by assuming their function. Consideration of the weights of these models and a further study of the areas of camera lucida projections of whole mounts of the thyroid glands of the host animals fail to show that they are markedly retarded in their growth by the transplants.

SUMMARY

1. Transplants of hypophysis, hyoid cartilage, oral epithelium, and thyroid anlagen undergo self-differentiation in an altogether abnormal position under the skin of the top of the head.

2. The thyroid anlage divides into right and left glands only when there is a hyoid keel upon which to divide; and it will envelop imperfect rod-like cartilage masses.

3. In certain cases the transplanted thyroid anlagen attained marked size, in one instance approaching the combined size of the host's own pair of thyroid glands. In this set of experiments it never caused any great retardation in the growth of the host's thyroid glands.

4. The pars intermedia of transplants added sufficient secretion to that of the host's own gland to produce visible effects, but this has not thus far been the case with the pars anterior of the transplanted hypophysis or with the transplanted thyroid gland. They have not as yet been shown to have an effect upon size growth or metamorphosis.

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XVII. THE METHOD OF THE THERMAL GRADIENT: ILLUSTRATED BY EXPERIMENTS ON THE EGG OF A MARINE INVERTEBRATE

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In the following I wish to suggest and to illustrate the usefulness of the thermal gradient as a means of analyzing the mechanics of early development.

One learns little concerning casual relations by merely observing the sequences of normal development. To discover forces and processes one must experiment; that is, he must manipulate his material in some precise and reproducible manner; and then he must analyze and correlate the results which he obtains. Now it is unfortunately true that most methods of experimental manipulation are violent; to a greater or less degree they inflict injury on the living material. Hence the results which they produce often show weakness and retardation. And even if weakening be avoided, still it is commonly true that secondary and regulative processes are awakened and some degree of reconstitution takes place.

The method of the thermal gradient has the advantage of not being violent. So long as the limits of temperature toleration of the organism in question are not exceeded, it does not subject the material to any unaccustomed stimulus. Furthermore, there is nothing in the nature of the method itself to call forth secondary or regulative processes. Work on amphibian development has clearly shown, however, that considerable regulation does occur after the heat gradient has been discontinued.

Most of the experiments in which use has been made of thermal gradients have been done within the last three years upon the early development of amphibia. I refer to work undertaken independently by Huxley (9) and by Dean, Shaw, and Tazelaar (4) in London, by Vogt (10, 11) in Munich, and by myself (5, 6) in Claremont. The results obtained have been in fair accord, and are to a considerable extent supplementary to one another. Briefly, they show that temperature differentials may affect the relative sizes of cleavage cells, the position and extent of the movements of gastrulation, the size and position and symmetry of the area which differentiates as neural plate, and the size and position of eyes, gills, limb buds, etc. For the purposes of the present symposium, however, I have thought it best to pass this amphibian work by and to confine my paper to the consideration of a few experiments performed this past summer on the eggs of a marine invertebrate, *Urechis caupo*, an Echiurid. The work was done at the Hopkins Marine Station, Pacific Grove, Cali-

fornia. I am most grateful to the staff of this station for the hospitality and for the many kindnesses which I received at their hands.

The eggs of *Urechis* are small: about 0.12 millimeter in diameter. In order to manipulate them a method had to be devised which was different from that used with amphibian material. The method which finally proved most successful involved sucking newly fertilized eggs into a capillary glass tube, and then placing the tube with the contained eggs between two hollow metal thermodes. By circulating water of different temperatures through the thermodes a fall of temperature of 30° Centigrade was established and maintained across one millimeter of distance. The difference of temperature between the opposite surfaces of the eggs is not accurately known, but was, quite likely, in the neighborhood of 3 degrees. To support the eggs in place within the capillary, and to prevent convection, they were imbedded in a thin agar gel. There was also gel between the capillary tube and the thermodes. The entire apparatus was placed on the stage of a microscope, covered with a cover glass, and observed by transmitted light.

In certain of the experiments a few eggs were kept continuously in view, and notes and drawings were made as the fertilization membrane rose, the polar bodies were given off, and the first two or three cleavages took place. Certain of the internal activities, such as the streaming of the contents of the germinal vesicle to the periphery, the formation and elongation of the maturation spindle, and the elongation and division of the mitotic spindles of cleavage could also be followed. These experiments convinced me that eggs properly imbedded in gel within the tube neither rotate nor shift in position, even when subjected to a steep temperature gradient. In another experiment the capillary tube and the thermodes were continuously moved back and forth in the field of vision, and repeated observations and rapid sketches were made of sixty-eight eggs, from the time the fertilization membranes first rose until the eggs were in the eight-cell stage.

I. THE INFLUENCE OF THE THERMAL GRADIENT ON POLARITY

A first result of differential warming was noted soon after heat flow had been established, namely, that the fertilization membrane rose more rapidly and attained a greater height on the warmed side of the egg (Fig. 2). Thus the protoplast came to occupy an eccentric position within the membrane, being nearer the cooled side. Incidentally, this observation suggests that the perivitelline space is at first a gel, else the position stated would not be a stable one.

A second observation, which became more and more evident as the time for the extrusion of the polar bodies approached, was that the protoplast was becoming flattened on the warmed side. To a less degree a

similar flattening sometimes appeared on the cooled side. Thus the cell, at first spherical, came to be an oblate spheroid with its short axis in the direction of heat flow. That this is actually what happened, and not an optical illusion due to the fact that the eggs were seen through the walls of a capillary tube, is evident from the consideration that the inclosing vitelline membrane continued to appear spherical. (See Fig. 2. In all the drawings the right side is the warmed side; the left, the cooled side.)

The significance of the observation cannot as yet be stated with assurance. I would suggest, however, that the thermal differential may have decreased the viscosity of the protoplasm of the warmed area and thus have permitted surface tension to be more effective on this side. At any rate, such an explanation would be in accord with most studies of the temperature coefficients of the viscosity of protoplasm. In connection with the observation that a slight flattening may occur on the cooled side, it should be noted that Heilbrunn (7, 8) by means of centrifuging experiments, found that the viscosity of the protoplasm of certain marine eggs is highest around 15° Centigrade.

A third observation is that the polar bodies tend to appear with greater frequency on the warmed side of the egg (Fig. 3). There were, however, exceptions to the rule, and it was not until the matter was analyzed statistically that the true relation between the localized warming and the place of extrusion of polar bodies was established. The results are summarized in the schematic Figure 1. The eggs have been grouped into twelve groups according to the orientation of the polar bodies with respect to the direction of heat flow. In all, forty eggs gave off polar bodies on the warmed side (right), fourteen gave them off in a neutral zone, and only six extruded them on the cooled side (left). The positions of the polar

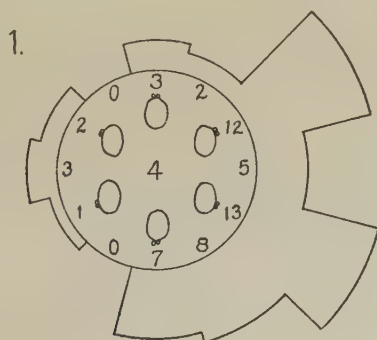
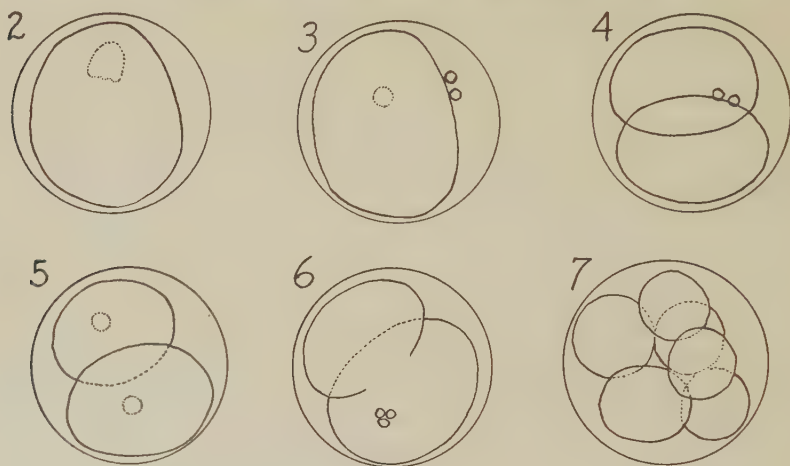


FIG. 1

FIG. 1.—Schema showing positions of the polar bodies. Heat flow was from right to left. By far the greater number of polar bodies appeared on the warmed side (right) of the eggs. In four instances the polar bodies appeared above or below.

bodies of eight eggs are unrecorded. In controls the polar bodies appear in the center of a somewhat flattened area. This was usually but not always the case in the gradient-treated eggs.

Figure 1 shows a further feature of interest. It will be noted that the decrease in the incidence of polar bodies is chiefly in the region 60 degrees removed from the cooled side of the egg. The great increase in polar bodies is in the region 30 degrees from the warmed side. These relations will be understood if we think of the effect of the thermal gradient somewhat after the manner of a force impelling the polar bodies from the cooled toward the warmed side. Only that component of the force is effective, however, which is parallel to the surface of the egg. Hence it is that the polar bodies most affected are those which normally appear in the regions at right angles to the direction of heat flow.



FIGS. 2-7.—Drawings of gradient-treated eggs. FIG. 2.—The fertilization membrane has risen higher on the warmed side (right) of the egg. The egg is also flattened on this side. The germinal vesicle has just broken down and its contents are streaming toward one side of the egg. FIG. 3.—The polar bodies have appeared on the warmed side of an egg. FIG. 4.—A first cleavage plane has developed nearly parallel to the direction of heat flow. FIG. 5.—Unequal division at the first cleavage. The warmed blastomere (right) is the larger. FIG. 6.—The polar bodies (on under side of the egg) are not in the plane of the first cleavage. FIG. 7.—At the third cleavage the warmed blastomeres have divided first.

It is evident that the temperature differential significantly affected the forces of the surface and those within the egg, thus altering the form of the protoplast and modifying the direction of streaming which carried the egg nucleus to the periphery. Perhaps the important change consisted, as already noted, in a lowering of the viscosity on the warmed side. The contents of the germinal vesicle, taking presumably the path of least

resistance in reaching the surface of the egg, would hence be strongly influenced toward the warmed side.

The experiment, furthermore, gives some basis for the belief that the polarity of the egg was modified. Now polarity is doubtless present in the egg of *Urechis*, as in most other eggs, before fertilization has been effected. Indeed, there is a certain cupping of one side visible in the unfertilized egg which may tentatively be held to mark the animal pole. To what extent there is previous to fertilization also a polarized arrangement of materials within the egg has not, to my knowledge, been established. By analogy with similar eggs, we may suppose that axial stratification arises mainly following fertilization, as a consequence of the active streaming of germinal-vesicle material toward the pole of the egg. If further work should bear out this assumption, then it seems reasonable to believe that the temperature gradient, by shifting the position of the polar bodies, also partially controls the distribution of stuffs within the fertilized egg.

It is clear that the gradient only partially controls such polarity, however. Other things being equal, the innate polarity of the unfertilized egg would be expected to pass over into the definitive polarity of the egg which has been fertilized. It has prospective value. But in the thermal gradient experiments other things are not equal. The effect of the gradient is not to be thought of as secondary and regulative in its nature, as though it reverses something already present and complete. Rather, it molds or bends something in process of development, something which is hence labile and incomplete. This distinction is of considerable importance in the theory of early differentiation.

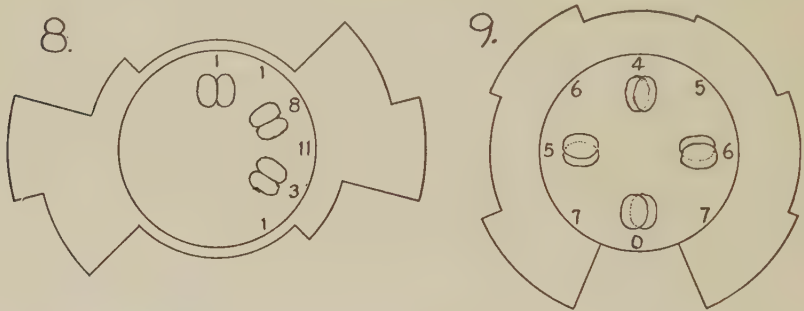
II. THE INFLUENCE OF THE THERMAL GRADIENT ON EARLY CLEAVAGE

The first cleavage spindle of eggs subjected to a temperature differential showed a strong tendency to elongate preperpendicularly to the direction of heat flow. As a result, the first cleavage plane tended to be parallel to the thermal gradient (Fig. 4). These facts were fairly evident in the descriptive experiments, in which a few eggs were kept continuously under observation. Indeed, in a few instances my drawings show a rotation of the cleavage spindle during its elongation, from a position nearly parallel to heat flow to a position almost at right angles to it.

The relation between the gradient and the plane of cleavage stands out clearly in the statistical treatment (Figs. 8 and 9). Considering first the 23 eggs in which the first cleavage plane was vertical with respect to gravity, we find that in one case the plane was perpendicular to the gradient, in 13 cases it was oblique to the gradient, and in 11 cases it was parallel to the gradient. Among the 40 eggs in which the plane was sloping with respect to gravity, we find four cases in which the horizontal axis of the plane was normal to heat flow, 25 cases in which it was oblique, and

11 cases in which it was parallel. In five eggs the first cleavage plane was horizontal and hence parallel to heat flow. In all, there was only one egg in which the plane of first cleavage was normal to the gradient, there were 39 eggs in which it was oblique, and 28 in which it was parallel. I have been interested to learn from Dr. Titlebaum, that in thermal experiments on the eggs of *Nereis* which he performed this summer at Woods Hole, he noted and recorded just such a shifting of the cleavage planes as I have described.

We may perhaps relate the observed change in direction of the cleavage spindle to the compressed shape of the protoplast which has been already described. Such an explanation would be in accord with Hertwig's so-called law of cleavage, and in harmony with the results of experiments in which eggs have been compressed between glass plates. Somewhat against this interpretation, however, is the observation that the oblate shape is less marked at the time of cleavage than it is a few minutes earlier when the polar bodies are given off. Another possible explanation is that some isothermic zone of the egg supplies optimum conditions for the



FIGS. 8 AND 9.—Schemas showing the directions of the first cleavage planes. Heat flow was from right to left. FIG. 8.—The directions of the cleavage planes in the 25 eggs in which the planes were vertical. FIG. 9.—The directions of the planes in the 40 eggs in which the planes were sloping with respect to gravity. In five eggs the planes were horizontal.

development of the spindle, the development of asters being possibly favored by a region of a certain viscosity.

A second effect of the thermal gradient on cleavage of the *Urechis* egg has to do with the relative sizes of the daughter blastomeres. In numerous instances the warmed blastomeres were larger; in no instance was a warmed blastomere smaller (Fig. 5). In the one case in which the first cleavage plane was perpendicular to the gradient, the warmed blastomere was larger. In 21 of the 39 cases in which the plane was oblique, the warmed blastomere was larger. In only one case of the 28 in which

the planes were parallel to heat flow was there a difference in the relative sizes of the first two blastomeres noted. The figures for the second cleavage are even more convincing. In 11 of the 12 cases in which the second cleavage plane was perpendicular to heat flow, the warmed blastomeres were clearly larger. The same was true of seven of the 31 cases in which the plane was oblique. In none of the 18 cases in which the plane was parallel was any inequality noted.

Tentatively we may attribute this difference in cell size to the effect of temperature on the rate of growth of the asters at the poles of the mitotic spindles. Chambers (1, 2, 3) has shown that each aster is a growing sphere of gel. It is reasonable to suppose that the aster of the warmed side will grow faster and therefore attain a greater size at the time when the cleavage furrow cuts across the egg. The matter is one of considerable interest in that it may illustrate how retardations in the rate of growth of asters from other causes, for example from internal differences in stuffs or in relative viscosities, may affect the sizes of cleavage cells. It is also possible that the thermal differential may have affected the positions of the asters indirectly, the greater viscosity toward the cold side favoring the formation of asters in this direction.

It is of interest that the pattern of cleavage as seen in the direction of the cleavage planes has not always agreed in normal fashion with the pattern of symmetry of the egg as indicated by the position of the polar bodies. Thus, in quite a number of instances the cleavage planes did not cut at all close to the polar bodies. (See Fig. 6. The polar bodies in this figure are on the under side of the egg.) The matter is not easy to observe, and the observations are perhaps not conclusive, but the evidence points to the likelihood that the factors which determine the position of polar bodies (and therefore presumably the arrangement of materials) are capable of being influenced independently from the factors which determine the direction of cleavage planes.

Such an independence of cleavage and symmetry would be less surprising were we discussing eggs, like those of the frog or chick, in which the cleavage planes are known to be variable and irregular and yet normal embryos result. But the egg of *Urechis* belongs to that group of eggs which are said to be of determinate cleavage, and in which, given normal development, the pattern of cleavage is closely related to the distribution of materials within the egg and to the pattern of the later embryo. Such being the case it is of considerable interest that the gradient should have affected the two patterns differently.

A third effect of thermal differentials on the cleavage has to do with the time sequence of the cleavages. It was observed that blastomeres of the warmed side of the egg, especially at the four-cell stage, divide just a little before those of the cooled side (Fig. 7). This is so in accord with

what one would expect under the circumstances that further comment at the present time is unnecessary. Huxley (9) has recorded similar relations in the rate of cleavage of frog's eggs subjected to temperature gradients.

The influence of thermal gradients on the chemical differentiation of primordia, in the case of the *Urechus* egg, has not yet been demonstrated. There are difficulties of technique in the way of studying this problem which have not yet been overcome. I anticipate that there will be found a profound effect, comparable to the considerable influence which Huxley, Vogt, and I have demonstrated on the amphibian egg.

The experiments which have been described indicate the usefulness of the method of thermal gradients as a tool for the analysis of early development. The method is not independent in the sense that it can of itself yield explanations of the phenomena which it discovers. On the contrary, it must lean heavily on other methods of study for interpretations. Its usefulness, as is the case with quantitative methods in general, would seem to be that it may serve as a check on interpretations and hypotheses arrived at by other methods.

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XVIII. OBSERVATIONS ON SOME SIPHONACEOUS GREEN ALGAE OF THE MONTEREY PENINSULA

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Practically all algologists are in agreement that the plants known as the algae are not a homogeneous monophyletic series. The three classes of algae considered in this afternoon's symposium have not originated from a common ancestry. The problem of determining the origin of the red and the brown algae is extremely difficult, since the simplest known forms among these algae have a rather complex organization, and connecting links between them and a hypothetical unicellular ancestor are unknown. One explanation of this absence of primitive reds and browns is that they arose at a time when the ocean was much less saline than at present and that there was a dying off of the more primitive forms as the salinity of the ocean increased. There was, however, a survival of certain more advanced forms in the salt water, and it is from these that we have the multitude of marine red and brown algae which, in turn, are characterized by an even greater complexity of organization. It is very doubtful, in spite of the arguments advanced by Church, if these marine algae have ever become adapted to a terrestrial mode of life and so served as the forerunners of the present-day land flora.

Evolution among the red and brown algae has been along the line of new combinations of cells into plant bodies of varying form, and along the line of specialization of sexual reproduction and spore formation. There has been no particular modification of the fundamental structural unit, the cell; and, in a very general way, one can say that the primitive type, the uninucleate cell, persists throughout both the red and the brown algae.

The evolutionary situation is quite different in the case of the green algae, and it is possible to trace them back to a primitive, motile, unicellular ancestor. Most algologists hold that this unicellular organism is of the *Chlamydomonas* type, and that the Volvocales are the lowest of the Chlorophyceae, or green algae. Next above the Volvocales stand the Chlorococcales, which include both unicellular and colonial non-filamentous forms. In both cases the vegetative cells are immobile. It is interesting to note that the Volvocales and the Chlorococcales are predominantly fresh-water in habit. While many of these simpler forms are probably of recent origin, some of them are undoubtedly quite similar to green algae of the ancient seas. Their persistence is probably due to their early migration from the ocean to the land and their ability to live in small temporary bodies of water or even on damp soil. The general lack of primitive green

algae in the present-day oceans may be accounted for by the inability of the great majority of them to endure the condition of increased salinity.

Several cell types have been evolved within the limits of the rather heterogeneous order of the Chlorococcales. Many genera of the order have uninucleate cells and a single cup-shaped chloroplast. In these, then, there has been a persistence of the Chlamydomonad type of cellular organization. Others of the Chlorococcales are characterized by multinucleate cells. The number of nuclei may be small during the vegetative stages of the cell's life, and may then suddenly increase just prior to reproduction, as in *Tetraedron* (12) and *Pediastrum* (11); or, the number of nuclei in the cell may continually increase as the cell grows older. Instances of the latter type of cell are to be seen in *Characium* (10) and *Hydrodictyon* (14). The remote relationship between *Characium* and *Hydrodictyon* shows that this multinucleate habit has been independently developed in different lines of evolution within the Chlorococcales. This multinucleate habit may be found in all members of a family, as in the Hydrodictyaceae, or there may be both uninucleate and multinucleate members within the same family. Examples of the latter are seen in *Chlorochytrium* (1), and in *Characium*, both of which are placed in the same family.

Although the multinucleate habit has not become firmly established in evolutionary sequences within the Chlorococcales it has become firmly fixed in a higher order of the green algae, the Siphonales. These algae have a plant body consisting of a single tubular multinucleate cell which, according to the genus, is of microscopic size and sparsely branched, or profusely branched and with the branches interwoven into a plant body of large size and definite shape. Sometimes, it is true, such large cells develop transverse plugs that make the plant multicellular, but such cross walls are quite different from cross walls in filamentous algae.

The great majority of Siphonales are found in the ocean, especially in tropical and subtropical waters. The temperature of the Pacific Ocean in the region of the Monterey Peninsula cannot, even by the farthest stretch of the imagination, be called tropical or subtropical. Nevertheless there are representatives of four of the six families of the Siphonales among the marine algae of the Monterey region.

Before discussing certain of these in detail, it might be well to consider the possible origin of the Siphonales. Beginning with such multinucleate Chlorococcales as *Characium* and *Codiolum* it is not much of a jump to the terrestrial fresh-water alga, *Protosiphon*. This alga, known largely from the work of Klebs, has been recorded two or three times in this country, but Moore and Carter (6) think that it is a rather widely distributed terrestrial alga in the United States. The single multinucleate cell of *Protosiphon* has a tubular, colorless, rhizoidal portion, which grows into the soil, and an expanded, green, bladder-like portion above ground.

Reproduction takes place by the repeated division of the whole protoplast, or only that in the aerial portion, into biciliate gametes, which are liberated by the gelatinization of the apical portion of the old mother-cell wall. The gametes unite to form thick-walled, lobed zygotes, which eventually grow into new plants. The family of the Protosiphonaceae, which includes a few algae similar in structure to *Protosiphon*, is placed in the Chlorococcales by some algologists, Fritsch and West (3), Oltmanns (7), Printz (8). Other algologists, West (16), Setchell and Gardner (9), consider this family the most primitive of the Siphonales. In my opinion it is more logical to think it one of the Chlorococcales looking toward the Siphonales than belonging to the Siphonales. In either case, however, it shows that the Siphonales have probably arisen directly from certain of the multinucleate Chlorococcales.

As far as the general structure of the plant body is concerned the marine alga *Halicystis* appears to be very closely related to *Protosiphon*. The best-known species of the genus, *H. ovalis* (Lyngb.) Aresch., has a plant body consisting of a short, rhizoidal, colorless portion and a dark green, subglobular portion above the rhizoid (Fig. 1, A). Mature plants

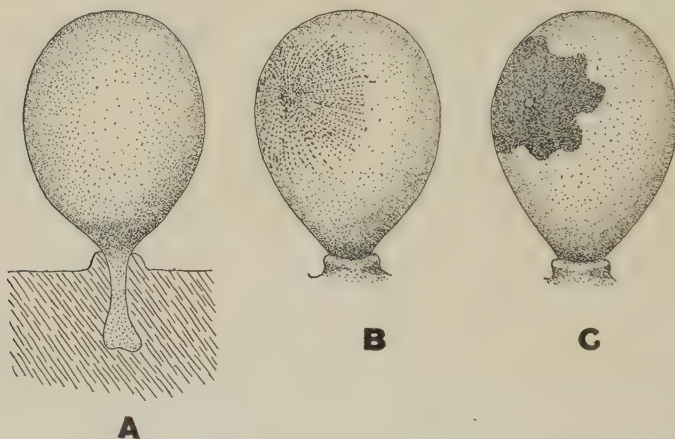


FIG. 1.—*Halicystis ovalis* (Lyng.) Aresch. A, vegetative cell showing the rhizoidal attachment to the substrate; B, beginning of the formation of a sex organ; C, cell with nearly mature sex organ.

may have the green portion as much as 15 mm. in diameter. According to Setchell and Gardner (9) this alga is extremely rare on the Pacific Coast of North America and they record it only from the Monterey Peninsula and from Vancouver, B.C. A survey made by G. J. Hollenberg the past summer shows that this alga is of much wider distribution on the Monterey Peninsula than previously supposed and that it is to be found all the way from Station Point (Point Cabrillo) to Point Lobos, wherever conditions

for growth are favorable. The alga grows best in clefts in rocks of sheltered tide pools and at levels which are exposed only by the lowest tides. It is always found growing upon and firmly attached to the thalli of crustaceous coralline algae.

The structure and development of *Halicystis* are known only from the work of Kuckuck (5) and the following account, partially in confirmation and partially in denial of his observations is largely based upon studies made by Mr. Hallenberg during the summer of 1929. Under the natural conditions of the Monterey Peninsula the plants are almost always sterile, but when they are taken to the laboratory and kept in running sea water they generally reproduce within from 5 to 6 days.

As has been shown by Kuckuck (5), the first indication of reproduction is an orientation of certain of the chloroplasts in double or single rows around light-colored areas in the protoplast (Fig. 1, B). These light-colored spots, which eventually become the exit pores of motile cells, lie in a transverse belt encircling the globular portion of the vegetative cell. Internal to the pore-bearing zone there is, according to the type of plant, the formation of a dense light- or dark-colored area in the protoplast (Fig. 1, C) and eventually a division of this portion of the protoplast into biciliate bodies. Attempts to prepare serial paraffine sections of plants at this stage of development have so far proved unsuccessful. However, it is evident from the observation of living material that these sporange-like areas are not cut off from the rest of the cell by definite walls but are merely separated by plasma membranes.

The Monterey material shows the same types of zoöspores as recorded by Kuckuck (5). The plants with dark-colored, dense areas produce biciliate pyriform zoöspores each of which contains many chloroplasts. Plants with light-colored, dense areas in the protoplasm give rise to smaller biciliate zoöspores, each of which contains but a single chloroplast. Kuckuck called those with many chloroplasts "macrozoöspores." The smaller ones containing but a single chloroplast he called "microzoöspores." He thought the macrozoöspores asexual reproductive bodies; the microzoöspores were also considered asexual in nature, but he thought that they might at times function as gametes and fuse with one another.

It was soon noted in the cultures of the Monterey *Halicystis* that plants which had contained sori the day before were often without them the following morning. Suspecting that there might be the same daily periodicity in reproduction as is found in *Pediastrum* and in *Hydrodictyon*, we placed the cultures in a dark room and then watched continuously from the time they were brought out into the light the following morning. Within a few minutes after the plants were brought into the light there was an abundant discharge of zoöspores from such plants as had mature sori. Usually the zoöspores were slowly discharged as a greenish mass

that dripped from the exit pores and slid to the bottom of the culture dish. Soon after reaching the bottom of the dish the mass became lighter and lighter in color as the individual spores in it swam away in all directions through the water.

In the case of one plant there was a forcible and sudden instead of a gradual discharge of the spores. Here, the zoöspores were discharged as a dark green jet that extended to some 10 mm. from the exit pore (Fig. 2, A). The jet dispersed much like a puff of smoke and within a minute after the green stream was ejected all traces of the spore mass had disappeared. Two or three minutes later there was a second jet-like discharge of zoöspores. This mass also dispersed very rapidly. The rapid

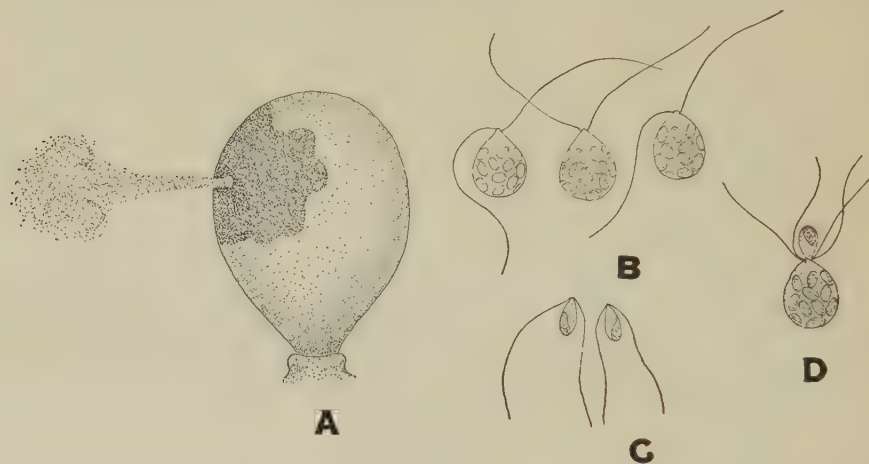


FIG. 2.—*Halicystis ovalis* (Lyng.) Aresch. A, showing the method of discharge of gametes; B, macrogametes; C, microgametes; D, union of gametes.

dispersion following ejection is ascribed to minute water currents set up by the discharge rather than to the spores moving away by action of their cilia. It is impossible to state whether or not the explosive discharge is more general than the gradual discharge of the spore mass, since the forcible discharge takes place so quickly that it is often overlooked.

The macrozoöspores (Fig. 2, B) and microzoöspores (Fig. 2, C) of *Halicystis* are so similar in appearance to the motile gametes of many other Siphonales that we were not content to accept Kuckuck's statement (5) that the two do not fuse when mixed with one another. Macrozoöspores swim for some time after discharge; microzoöspores swim for a short time only. We were therefore particularly careful to obtain freshly discharged zoöspores of both types before mixing them together. When freshly discharged spores were mixed there were repeated cases of the fusion of the two. It is obvious, therefore, that Kuckuck was incorrect in interpreting

these as zoöspores. The motile cells of *Halicystis* are gametes, and with the same marked heterogamy as is found in *Bryopsis* and in *Codium*.

In one series of experiments the small male gametes swarmed vigorously around the various large female gametes and gathered in clumps because of the entangling of their cilia. Such a gathering of many male gametes around a single female motile gamete is quite similar to that found in *Ectocarpus*. In other cases a single free-swimming male gamete united with a motile female gamete. Such fusions usually took place by the two becoming mutually apposed at their hyaline anterior ends (Fig. 2, D). Sometimes, however, the two were lying side by side but with their apices joined to each other. In these cases there may have been an axial apposition and then a bending back of the male gamete.

Although we have fully demonstrated that the gametes unite with each other, the observations in the further history of the zygote are as yet but fragmentary. The imperfect technique of cultivating gametes and zygotes, as well as lack of time, has not as yet given new plants from reproductive stages of *Halicystis*. Certain experiments, however, are quite suggestive. Three culture series were started at the same time, one containing male gametes only, another containing only female gametes, and a third containing both types of gametes. Slides were placed in the culture dish so that such motile cells as came to rest upon them could be repeatedly examined microscopically without disturbing the substrate on which the cells had come to rest. In the cultures containing only male gametes or only female gametes the cells that had come to rest never developed a firm outline, and within two or three days they began to show signs of disintegration. In the cultures in which male and female gametes had been mixed there were small rounded cells, about the size of female gametes, which had a definite clean-cut outline, and the chloroplasts at one side of the cell. Other cells in the culture showed the disintegration phenomena observed in the other cultures. The persistent cells have been interpreted as zygotes, the others as disintegrating gametes. The subsequent fate of the zygotes has not been followed.

The foregoing demonstration of a heterogamous sexual reproduction shows that *Halicystis* should be removed from the Protosiphonaceae and made the type of a new family, the Halicystaceae. This family is the most primitive of the Siphonales and is primarily distinguished from the other families by the fact that the gametanges are not formed in special lateral outgrowths from the plant body.

Codium, another genus of the Siphonales, has a vegetative structure quite different from that of *Halicystis*. The single cell constituting the *Codium* plant is tubular in shape and is repeatedly branched. In some of the species, among them *C. Setchellii* of the Monterey flora, the branching filaments are densely interwoven to form cushion-like masses, which grow

firmly attached to the rocks. The superficial portion of the cushion consists of enlarged ends of branches (utricles), which lie parallel to one another in a palisade-like manner. Other species, as *C. fragile* of the local flora, have a broad holdfast from which arise repeatedly and dichotomously branched cylindrical outgrowths, some 3 to 10 mm. in diameter and 25 to 40 cm. long. The cylindrical branches of this plant have a central core of densely interwoven threads and a superficial layer of palisade-like utricles (Fig. 3, A).

Reproductive structures were first noted by Thuret (13) in *C. tomentosum*, a species with much the same general organization as *C. fragile*. He showed that certain of the utricles produced one or two lateral, club-shaped outgrowths with dense protoplasts (Fig. 3, B) and that the contents of the outgrowths eventually became divided into numerous biciliate cells. These cells, which he considered zoöspores, were discharged in a mass by the rupture of the apex of the sporange. Later investigators found that there were two types of sporange in this species of *Codium* and that the two types were borne on separate plants. Some plants produced sporanges that were dark green in color and contained large biciliate cells. Other plants produced yellowish sporanges which contained small biciliate cells (Oltmanns, 7). These two types of motile cells function as gametes and there is a formation of new plants only when the two types of gametes are mixed. This interpretation of the motile cells of *Codium* has been universally accepted since then, but there have been certain investigators, notably Wendt (15), who think that the female gametes may also develop parthenogenetically into new plants.

Since the actual demonstration of the fusion of gametes was known only for *C. tomentosum*, a European species, studies were undertaken in the summer of 1926 to see how far the Pacific Coast species agree with those of Europe. The same idea had previously occurred to the algologists at the Puget Sound Station and Miss Hurd (4) attempted to work this out for the local *Codium fragile*. Although she found this species abundant in the Puget Sound region, all plants were of the same kind and contained female gametes only. This difficulty is not encountered on the Monterey Peninsula, since both male and female plants can be found in abundance and with mature gametes all through the months of July and August. Miss Hurd (4) ascribes the wide distribution of *C. fragile* in the Puget Sound region to a parthenogenetic development of female gametes, and dismisses the idea that this plant might form both types of gametes at another time of the year. The regular development of both male and female gametes in the Monterey region and their seasonal restriction to the summer months would seem to show that the presence of both male and female plants in the Puget Sound region cannot be denied until they have been observed throughout the entire year.

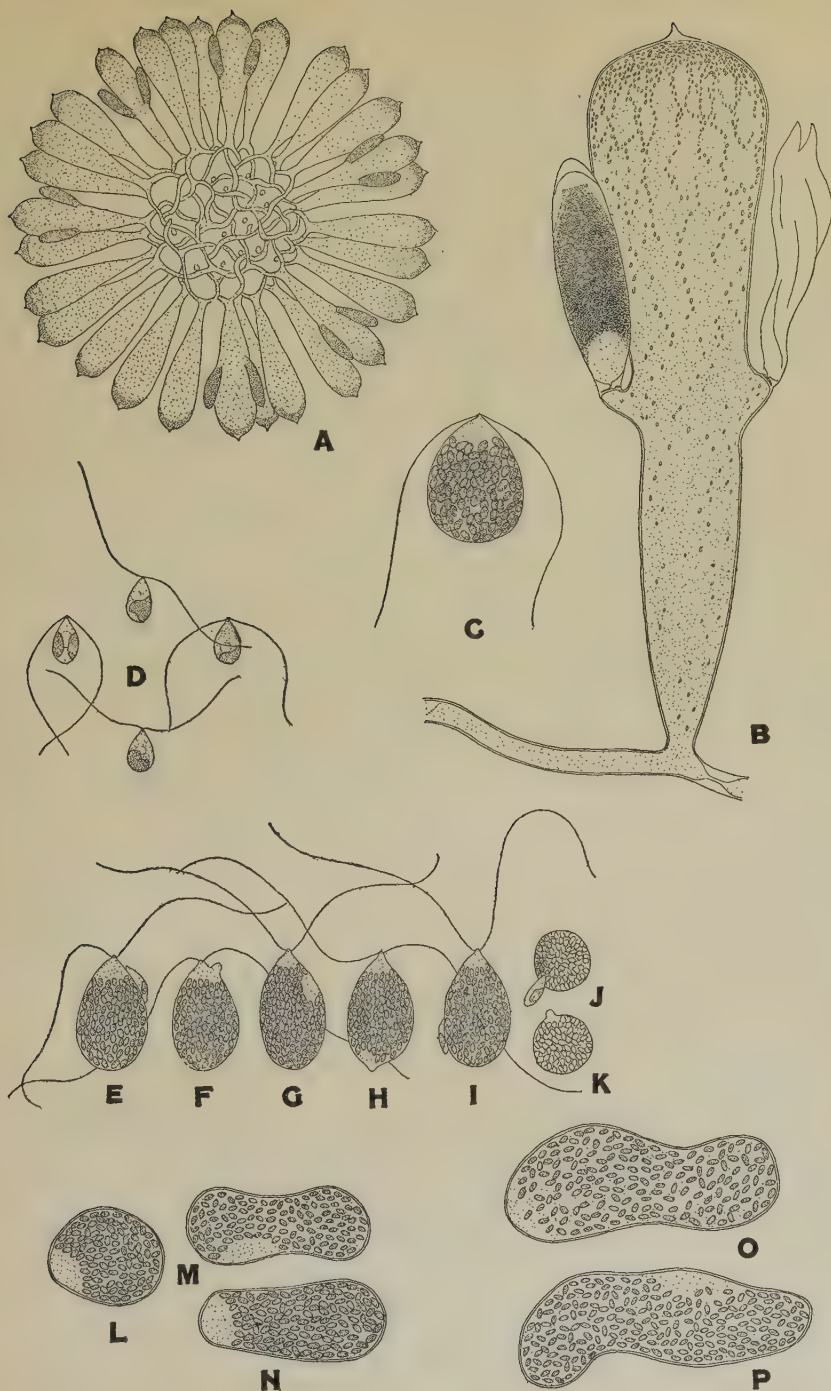


FIG. 3.—*Codium fragile* (Suring.) Hariot. *A*, diagrammatic transverse section of the plant body; *B*, utricle with an immature and an empty male sex organ; *C*, macrogamete; *D*, microgametes; *E-K*, stages in the fusion of gametes, *J* and *K* in vertical view; *L-N*, 6-day-old germlings; *O-P*, 11-day-old germlings.

Male and female plants of *Codium fragile* cannot be told apart by macroscopical examination, but they are easily differentiated under low powers of the microscope, even before the gametes are fully mature. Sex organs are usually formed only by plants that are 15 cm. or more in length and in a region some 2 to 20 mm. back from the tips of the various branches. One finds occasional plants, however, in which there is a production of sex organs throughout the entire plant. The maturation of the gametes within the sex organs is a slow process, and a random selection of 50 plants that have apparently mature gametes will seldom give more than 10 per cent discharging gametes.

The best method of obtaining a discharge of gametes is to allow the plants to remain in the air for from 5 to 8 hours after they have been uncovered by the tides and then cover them with sea water. Contact seems to accelerate the discharge, and the gametes will generally be found coming from such branches as have been pressing against the bottom of the dish. The discharge of gametes from each gametange occurs abruptly and is probably due to an imbibitional swelling of the material within the sex organ. The whole process, which takes but 30 seconds or so, begins with a rupture of the lid-like apical portion of the gametange. There is next the extrusion of a solid gelatinous mass that has much the same shape and size as the sex organ. Within the gelatinous vesicle is an axial canal, of smaller diameter than the female gametes, and the gametes move rapidly through this canal and accumulate in a heap at its free end. Movement of the gametes from the gametange and through the canal is purely passive and is due to hydrostatic pressure within the gametange. The passive nature of the gametes during their discharge is shown both by the lack of ciliary movement and by their being squeezed to a narrower diameter while being pushed through the canal. Cilia are soon evident on gametes that have moved through the canal, and within a minute or two after the gametes are discharged they begin swimming away from the heap at the end of the canal.

The structure of the gametes of *C. fragile* is identical with that of the European *C. tomentosum*. The female gamete is pyriform in shape and with two long cilia at the hyaline anterior end (Fig. 3, C). Within the median and posterior portions of the gamete are many small ovoid chloroplasts. Male gametes are about one-third the length and one-fifth the breadth of female gametes. They are more narrowly pyriform, are biciliate, and usually contain but a single chloroplast in the rounded posterior end (Fig. 3, D).

As far as could be determined the union between the two gametes is lateral, the small male gamete becoming applied to the side of the female, withdrawing its cilia, and then gradually fusing with the female gamete (Figs. 3, E-3, K). Shortly after the fusion of the two the zygote becomes

immobile and secretes a firm cell wall. The development of the zygote into a new plant is an extremely slow process, and in the three weeks during which the germination of the zygote was followed they did not grow to more than 4 to 5 times their original length. In the first week following gametic union the zygote has much the same general shape as when first formed and there is a hyaline region at one end (Figs. 3, L-3, N). As elongation begins, this region is still evident, either at one pore or on the side on the elongate cell into which the zygote grows. With continued growth this hyaline region becomes gradually obliterated because of the tendency of the chloroplasts to lie at a greater distance from one another (Figs. 3, O-3, P).

Results thus far obtained indicate that germination stages are found only in those cultures started by mixing the two types of gametes. These results seem to show that there is no parthenogenetic development of the female gametes, but the experiments have not been repeated a sufficient number of times to state positively that there is never a parthenogenetic development of female gametes.

There are no data at hand concerning the reproduction of the local species of *Bryopsis* (*B. corticulans* Setchell), since all collections made on the Monterey Peninsula have shown sterile material only. In view of what has been found with respect to *Codium* one would expect that this local species of *Bryopsis* would show the same union of motile heterogamous gametes as has been reported for the European *B. cupressoides*.

The three genera thus far discussed are exclusively marine. A fourth genus of the region, *Vaucheria*, is found in fresh-water and brackish habitats as well as in salt water. This alga is so familiar to all that little need be said concerning its structure and reproduction. As is well known, the tubular multinucleate cell may be either unbranched or with lateral branches. Even when branched the plant is extremely simple and never of the size attained by *Bryopsis* and *Codium*. From the standpoint of body structure it is among the simplest of the Siphonales. However, from the standpoint of sexual reproduction it is among the most advanced of the Siphonales, since the specialization of gametes has progressed to a state where there is a large immobile egg and a small motile antherozoid.

Setchell and Gardner state that they have made repeated collections of marine *Vaucheriae*, especially about San Francisco Bay, but they have found sterile material only and so cannot state what species are to be found in the Pacific Coast algal flora. It is particularly gratifying, therefore, that information is now available concerning the reproduction of one of the Pacific Coast marine species.

This material was collected from Elkhorn Slough, some fifteen miles north of the Hopkins Station. Certain of the filaments in this collection had greatly swollen ends, which were separated from the rest of the filament

by a transverse wall, and each contained a single rounded protoplasmic mass. These structures were at first interpreted as aplanospores, a well-known method of asexual reproduction in *Vaucheria*. However, they were so unlike the aplanospores of fresh-water species that a return trip was made to the Slough to collect as many specimens of this marine *Vaucheria* as possible. When the material was brought back to the laboratory each separate collection of the alga was placed in a covered glass dish containing a small amount of water and placed so that it would be favorably illuminated. Within a few days the majority of the filaments had grown or crawled out from the water and along the sides of the dish. In some of the cultures there were the same aplanospore-like bodies as were found in the first collection of this species. In other cultures many of the free ends of the filaments bore numerous short conical projections. The portions of the filaments with conical projections were always separated from the remainder of the filament by a short, colorless cell.

These structures at the ends of the filaments are the sex organs and not asexual reproductive bodies as at first supposed. The short empty cell separating the male sex organ (antherid) from the rest of the filament shows that the species belongs to the Piloboidae section of the genus. The only dioecious species in this section is *V. litorea*, a marine species found in the Atlantic Ocean. The local species is also dioecious, but is sufficiently different from *V. litorea* to be considered a new species.

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XIX. SOME QUESTIONS IN THE LIFE HISTORIES OF THE PHAEOPHYCEAE WITH PARTICULAR REFERENCE TO SCYTOSIPHON LOMENTARIUS

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We have a general knowledge of the haploid-diploid generations in the Phaeophyceae based mostly upon probable homologies with a few species which have been studied in detail. These species are fairly well distributed throughout the group of brown algae, and thus the homologies are probably largely correct. The accuracy of inferred homologies depends, of course, upon the closeness of the correspondence of the species compared and upon the details known of both. There is, however, a surprisingly large amount of inference in comparison with the relatively small amount of accurate observation concerning the union of gametes and concerning the reduction of the chromosome number. The observation of the union of gametes will not be taken up, but let us consider cursorily the observations on reduction.

If one accepts the grouping of Oltmanns (8), at least four of the seven orders have had reduction established in one or more species by actual observation. In the Ectocarpales and the Sphacelariales to our knowledge it has not been directly observed. These two lowest groups distinctly need such investigations. The Ectocarpales are especially basic in their morphology, because a great variety of forms of plants are interpreted in terms of these simpler members. In the Cutleriales it was first seen in *Cutleria* by Yamanouchi (15), and later in *Zanardinia* by the same author (13). In the Laminariales it was first reported as observed in *Chorda* by Kylin (4); later Myers (6) established the point of reduction in *Egregia*. In the Dictyotales Mottier (5) first definitely established the point of reduction in the tetrasporangium of *Dictyota*. In the Fucales this was located by Strasburger (14) in *Fucus*. It was later confirmed by Farmer and Williams (2), and with much detail by Yamanouchi (16). In the Tilopteridiales the actual point of reduction has not been found by direct observation, so far as we are aware, but it has been inferred by homology with other species in adjacent orders.

The observations are fairly recent, and while no complete list is attempted, they are quite few in any group. It follows that in tracing the life histories in the brown algae most of the homologies are derived by comparing whole plants of one genus with whole plants of another, or the form of sex organs in one with those of another, or the form of one sporangium with that of another. While these are undoubtedly correct in the main, they are sometimes doubtful. In any case a similarity of life history is inferred, which may not be true throughout; the larger the

amount of inference in comparison with the amount of observed detail, the less the certainty. We have reached the point where accurate detail of many brown algae distributed throughout the orders is necessary for accurate comparisons and for the determination of relations of genera to each other. In fact, we know of no group in the plant kingdom in which detailed morphology is so much needed as in the Phaeophyceae, and within the group, nowhere so much as in the Ectocarpales.

To give some idea of how little is known of the brown algae of the northwest coast of North America, one needs only to group the species, as given, for example, in Setchell and Gardner (13), according to their reported known life histories. These authors list approximately 250 species, which may be approximately grouped as given below. The figures cannot be exact because there is variability in the value of species; and it is difficult to set up a standard of what is really known, considering the gradation through all stages of inference. But the figures illustrate the point nevertheless.

In about 21 per cent of the species both haploid and diploid generations are reported on different individuals. In very few of these have they been demonstrated. In nearly all of them they have been inferred by apparent homology. Thus in about one species in five do we believe that both generations may be considered to be known.

In about 32 per cent only the diploid generation is known, or inferred, very largely the latter. Usually this means that the unilocular sporangium was observed, and, since no union of cells from unilocular sporangia has been seen, that we have here an asexual spore formation and that this is the point of reduction. The burden of evidence favors such a conclusion, but there is little evidence.

About 30 per cent are known only from the haploid generation. This means that multilocular reproductive organs, known in some cases to produce gametes, are inferred to do the same in an overwhelming majority of the cases.

In about 12 per cent no reproductive organs have been seen. Thus the generation is uncertain.

With roughly one-third of our browns with diploid generation unknown, and roughly one-third with the haploid one unknown, one naturally infers that some of these haploid species may prove to be the same as some of these diploid ones. This inference became a probability when Angst (1) cultivated *Ectocarpus* with multilocular reproductive organs from *Soranthera* with only unilocular reproductive organs. The unknown generation may be already known, but the connection may be the unknown feature.

One of the most disturbing observations is the occurrence of both unilocular and multilocular reproductive organs on the same individual. Inferentially these are, respectively, sporangia in which reduction occurs,

and gametangia. Are these haploid or diploid plants, or has there been an error of observation or of homology? About 5 per cent of our west coast Phaeophyceae are reported to have, at least sometimes, both forms of reproduction on the same plant body. Referring again to Setchell and Gardner's work (13), the list is as follows: *Pylaiella littoralis* (L.) Kjell.; *Ectocarpus confervoides variabiles* (Saunders) S. & G., *E. confervoides parvus* (Saunders) S. & G., *E. hemisphericus* Saunders (according to Saunders); *Streblonema investiens* (Collins) S. & G.; *Ralfsia clavata* (Carm.) Crouan (reported by Kuckuck in 1894), *R. verrucosa* (Aresch.) J. Agardh; *Gonidia johnstonii* S. & G., *G. marchantia* S. & G.; *Leathesia difformis* (L.) Aresch.; *Punctaria occidentalis* S. & G., *P. expansa* S. & G.

So far as we are aware, in not one of these has the number of chromosomes been reported. Naturally, such report would need to be followed by the growing of the plants produced by both kinds of reproductive bodies with a view to chromosome counts. It will be observed that all of these species fall within the Ectocarpales. This is remarkable and alone would indicate this order as most promising in results for investigation.

It is not yet established that all the brown algae have uniformly one form of bisexual or two forms of unisexual gametophyte. There may be more. Nor is it certain that there is always just one form of sporophyte. In the green algae the haploid generation is known to reproduce by means of zoöspores. It is remarkable that diploid zoöspores do not occur. Possibly they do occur in the lower Phaeophyceae, and account for some of the peculiar forms of plants grown from reproductive cells (Sauvageau, 11, Oltmanns, 7).

In the spring and summer of 1928 and of 1929 *Scytosiphon lomentarius*, one of the Ectocarpales with an incomplete life history, was grown at the Puget Sound Biological Station with a view to determining whether its development could be completed. It was thought that possibly the unknown diploid generation might prove to be a known plant whose haploid generation is not known. The results were not as surmised, and are not entirely clear; so further work is contemplated in the summer of 1930. However, the results are here given so far as observed, together with some comments, because they will illustrate our uncertain knowledge of the Ectocarpales.

The plants are fairly common about the mean low-tide line in the vicinity of the Puget Sound Biological Station. These were gathered in mature condition and brought to the laboratory, where they were usually kept out of water for several hours. The plants were then washed in sea water, and the darker regions were broken into pieces about 5 mm. in diameter. These were put into glass dishes containing glass slides arranged on the bottom, and thereto were added 3 to 5 cm. of sea water dipped directly from the ocean. The quantity of material put into the dish was

perhaps such that if spread it would cover about one-twentieth of the surface of the water. The amount of material naturally varied with the amount of reproduction on the fronds, but an excessive amount of material increased the infection of the culture with diatoms and protozoa, as well as covered the slides too thickly with reproductive cells. Sometimes the reproductive cells were shed immediately and profusely; at other times they were not shed for several hours. Undoubtedly conditions of ripeness, and perhaps of drying in the course of preparation, affected the rate of their escape.

Within twenty-four hours the slides were removed to other dishes containing a culture medium made up of sea water sterilized by boiling under ten pounds pressure for at least five minutes, into which was put, for every 500 cc. of water, a crystal of Na_2HPO_4 about the size of a grain of wheat, and twice that amount of NaNO_3 . Slides with thinly strewn reproductive cells on them were preferable. On thickly strewn ones the individuals soon grew so intertangled that specific plants could not be followed with certainty. Some experience here was necessary, but in general a slide had enough on it if when held up to the light it bore a slightly brown film just visible to the naked eye. These slides were placed on the bottom and set on end along the walls of the dish, care being taken that the solution covered at least the lower 5 cm. of a 7.5 cm. slide. The temperature of the culture was kept nearly that of the sea by setting the dishes in a tray about 7.5 cm. deep into which constantly flowed water that had run through a pipe in the sea and thence to a tap in the laboratory. Here the dishes were kept, with covers to prevent contamination by dust but slightly raised to admit air.

The cultures were examined at least once in twenty-four hours. This was done by removing the slide, wiping the under side, putting on a cover glass, and putting it directly on the stage of the microscope. About every ten or fifteen minutes, drops of the culture solution were put on the slide with a medicine dropper to keep it from drying. A slide could thus be kept under the microscope for several hours if not permitted to dry and if the room was cool so the plants remained cool. An oil-immersion lens was used without much difficulty for the finer work. The cover glass was easily slid off on the water beneath it before the slide was returned to the dish.

The reproductive cells, which are interpreted in literature as gametes, contained only one chloroplast and had two unequal cilia. There was some tendency toward grouping, as stated by Reinke (9), but fully half of them were scattered. Whether the tendency was natural or accidental was not clear. Those in groups were hard to follow in their development because they could not be seen separately. The cells were 3.5–5 μ wide and 5–8.5 μ long. The anterior half was usually colorless and contained one or two

translucent bodies similar to those which Sauvageau (11) interprets as pyrenoids. The chloroplast was parietal and in the posterior half. In the chloroplast or just at its periphery was a red "eye-spot." By permitting slow evaporation under a cover glass, one could thin the film of water until the reproductive cells slowed their movements to the point at which the cilia could readily be seen. They arose just posterior to the center of the cell, and the anterior one was two or three times as long as the posterior one (Figs. 1-2).

No union of these cells was observed, nor were any observed with two chloroplasts, as figured after union by Kuckuck (3). This causes us to doubt whether such union is usual, or, indeed, whether it really takes place at all. We did not, however, observe them at break of day, as Kuckuck (3) did. This it is proposed to do in 1930.

Development was not uniform, as might be expected; the average rate is given in the following account. The first day the cells rounded up and withdrew their cilia; the chloroplasts filled most of the cell; the eye-spots remained clearly visible (Figs. 3-4). The second day the cells put forth tubes from one-half to two-thirds the diameter of the cells in width; the eye-spots were still visible; the chloroplasts were slightly irregular parietal sheets; and there was only one to a cell (Figs. 5-6). A few formed the first transverse wall on the third day, and on the fourth day perhaps the average had done so (Figs. 7-9). On the sixth day the plants were filaments 2-4 cells long (Figs. 10-13).

Examination during the next few days showed additional growth in length and width of the filaments (Figs. 14-21). Some of them showed a rhizoid-like filament from the original cell. This was longer and narrower than the other part of the filament, with longer cells, and with the chloro-

EXPLANATION OF FIGS. 1-22

- FIG. 1.—Motile ciliate cell. $\times 1733$
 FIG. 2.—Ciliate cells becoming quiescent. $\times 1733$
 FIG. 3.—Reproductive cell after losing its cilia. $\times 1733$
 FIG. 4.—Group of attached reproductive cells before tube formation. $\times 833$
 FIG. 5.—Germinating reproductive cells 48 hours old. $\times 1733$
 FIG. 6.—Same as Figure 5. $\times 833$
 FIG. 7.—Two germinating reproductive cells 3 days old. $\times 833$
 FIGS. 8, 9.—Filaments 4 days old, one or two cells long. $\times 833$
 FIGS. 10, 11.—Filaments 5 days old. $\times 833$
 FIGS. 12, 13.—Filaments 6 days old, two or three cells long. $\times 833$
 FIGS. 14, 15.—Filaments 7 days old. $\times 833$
 FIGS. 16, 17.—Filaments 8 days old: *o*, oldest cell; *r*, rhizoid-like end. $\times 833$
 FIG. 18.—Filament of 9 days old, without rhizoid. $\times 833$
 FIG. 19.—Filament 11 days old: *o*, oldest cell, *r*, rhizoid-like end. $\times 833$
 FIGS. 20, 21.—Filaments 12-13 days old: *r*, rhizoid-like end; *o*, oldest cell. $\times 833$
 FIG. 22.—Filament 15 days old: *o*, oldest cell; *r*, rhizoid-like end. $\times 833$



plants elongated and the color much fainter. The plants were not all alike; some were long and narrow, others short and thick. Occasionally the older cells of the short thick plants showed a tendency to branch.

About the fourteenth day some of the cells at or near the tips of the thicker filaments had divided longitudinally, thus beginning plate formations or series of close lateral branches (Figs. 22-24). Some of the plants branched much more than others. Occasionally very small translucent bodies were seen at the edges of the chloroplasts. Here and there they were in motion, although there was no opening in the cell walls (Fig. 25).

During the period when the plants were from 17 to 59 days old, some of them, chiefly those less branched, lost the contents of some or all of their cells (Figs. 26-28). In this process the chloroplasts darkened gradually to a deeper brown, which then faded to a grayish white and appeared granular, except at one point where they turned dark red. Meanwhile the contents of the cells escaped through single holes in the walls. In some of the cells one or two small translucent swimming bodies were seen in the cell after the escape of the protoplasmic mass (Fig. 29).

The escaping mass remained near the cell, was never seen to move, and its slight change of location is presumed to be due to amoebic rather than ciliary locomotion. The red spot often divided into two or three parts. The grayish portion of the mass showed the granules or aggregations of protoplasm with increasing distinctness, and the granules showed a barely discernible darker spot within.

The translucent bodies probably swam away, although the actual moving away was not seen. Such moving bodies were abundant on the slides at this time and not before. The whole protoplasmic mass disappeared, except the red spot, and a trace of the original outline (Fig. 30). The red

EXPLANATION OF FIGS. 23-32

FIGS. 23, 24.—Filaments 18 days old, showing beginning of discoid growth: *r*, rhizoid-like ends; *o*, oldest cells. $\times 466$

FIG. 25.—Cells from filament 18 days old, showing translucent motile bodies, *t*. $\times 1733$

FIG. 26.—Cells from filament 19 days old, showing contents in process of extrusion. $\times 833$

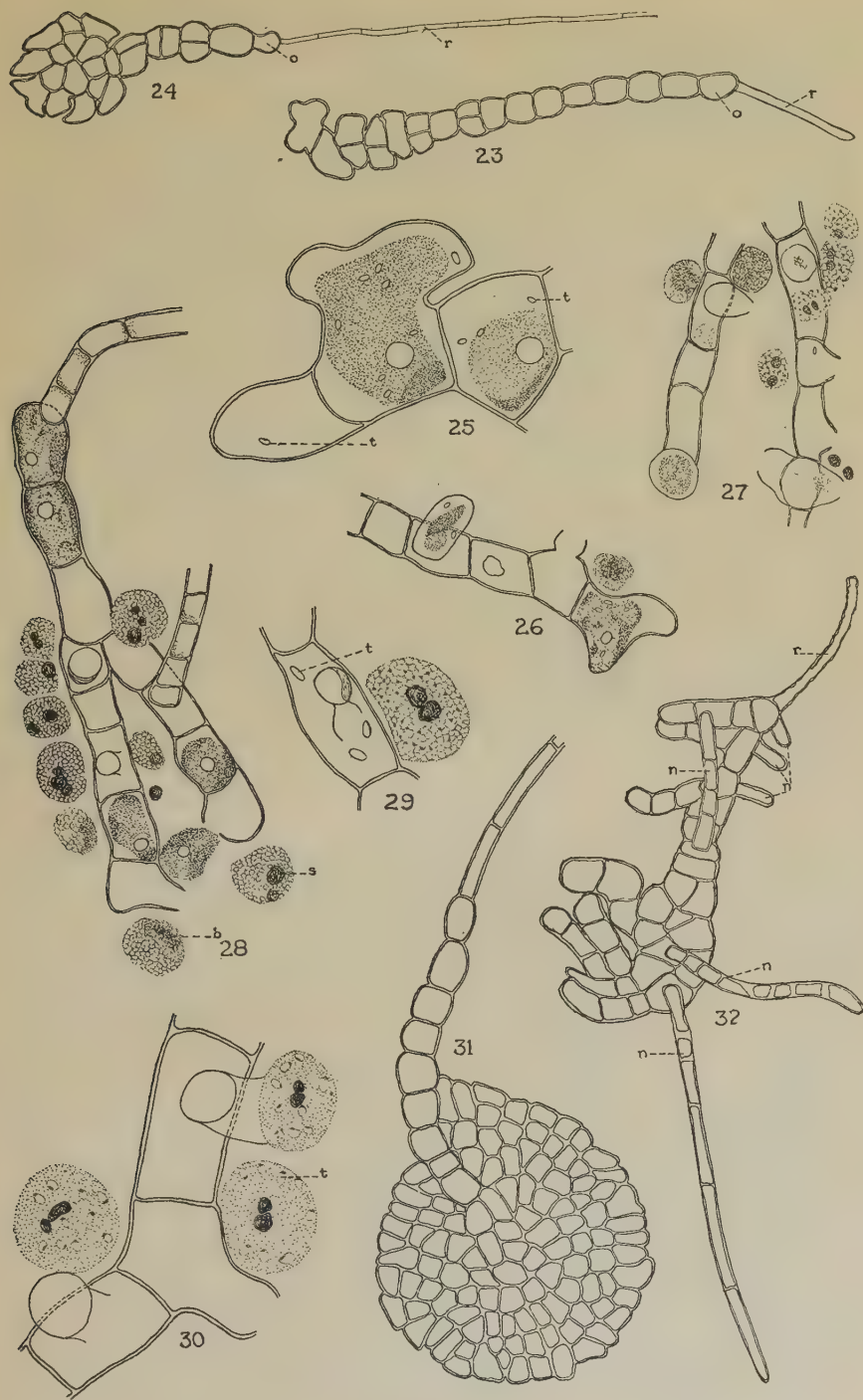
FIGS. 27, 28.—Stages in extrusion of cell contents, 19-22 days old: *b*, brown chloroplast; *s*, red spot; respectively, $\times 833$ and $\times 946$

FIG. 29.—Extruded cell contents: three translucent motile bodies, *t*, in old cell. $\times 1733$

FIG. 30.—Extruded cell contents with most of the translucent motile bodies, *t*, already escaped. $\times 1733$

FIG. 31.—Filament with well-developed discoid end, 22 days old. $\times 466$

FIG. 32.—Filament with probable new filaments, *n*, forming from the old cells; these new filaments seem to grow into the large well-known plant; *r*, the old rhizoid-like end. $\times 466$



spots disappeared within about three days after the escape, usually being eaten by unicellular animals.

Those plants which formed disks did not show this escape of cell contents. The disks became several cells thick in the middle (Fig. 31). These plants were not, however, sharply differentiated from those which formed no disks, in that one could pick out a series of gradually less well-formed disks grading into simple filaments (Fig. 32). Whether there was any sharp distinction between those which formed escaping cell contents and those which did not was not determined. Occasionally the same filament seemed to show characteristics of both kinds (Figs. 33-34).

About the twenty-seventh day new filaments appeared, almost always on the plants which showed no escaping cell contents. These at first resembled somewhat the rhizoid-like branches, but later had more nearly square cells, with a single, deep brown, more complex chloroplast which was less distinctly parietal. These filaments were much more vigorous than those which arose from the reproducing cells (gametes?), and grew much more rapidly. Usually several of these grew from a disk (Figs. 35-36). This is what Reinke (9) saw. The cells of these new filaments divided longitudinally as well as transversely, as Reinke states (Fig. 37). Their development was observed until they were several cells thick. Occasionally they branched, but the branches usually remained one cell thick and were thus hair-like (Fig. 38). This was the condition in 59 days. The original filaments had by this time almost disintegrated.

DISCUSSION

How shall we interpret the escaping cell contents? If Kuckuck (3) were in error in his observation of the union of gametes, then one would expect either the translucent bodies or the whole escaping contents to be a gamete, probably the male gamete. This seems the more likely in that (a) no growth occurred from any of the escaped contents so far as we could see, and (b) further growth of the filaments with the plate-like part

EXPLANATION OF FIGS. 33-39

FIG. 33.—Filament which seems to have both escaping contents, *e*, and new filaments, *n*, arising from it. $\times 466$

FIG. 34.—Portion of filament beginning formation of new filaments, *n*. $\times 833$

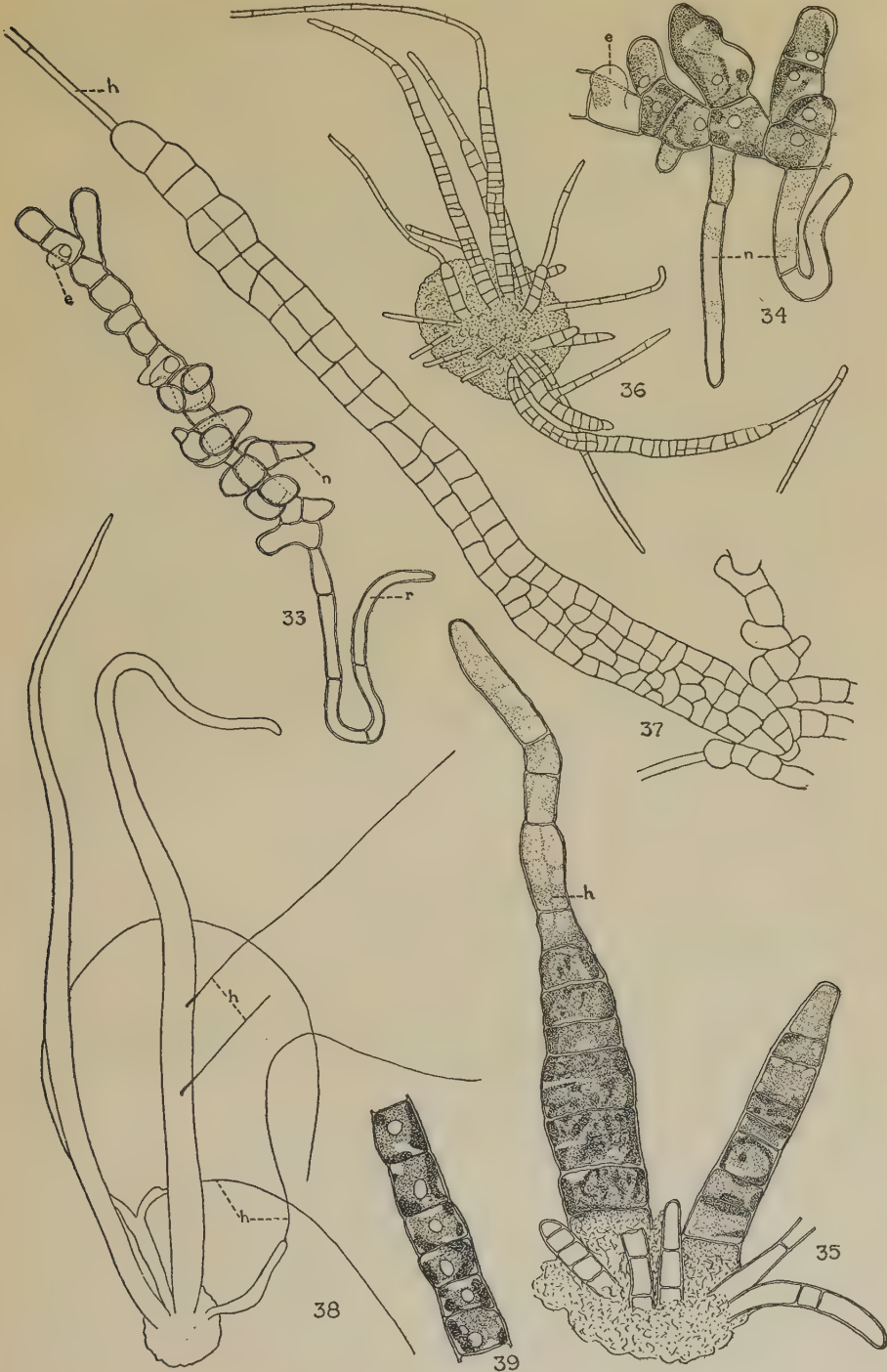
FIG. 35.—New filaments growing out of discoid filament 27 days old. The upper part of the largest is a hair-tip, *h*. $\times 833$

FIG. 36.—Group of the well-known plants arising from the discoid filament similar to that in Figure 31; 32 days old. $\times 190$

FIG. 37.—A new plant at 33 days: *h*, hair-like tip. This particular tip was 10 cells long. $\times 466$

FIG. 38.—Two of the largest plants found, 59 days old: *h*, hair-like tips. $\times 106$

FIG. 39.—Detail of cells near tip of 59-day-old plant. $\times 833$



always followed the escape of the cell contents from threads which were not plate-like. If the foregoing is correct we are not certain whether the whole escaped content is the gamete or whether the escaped content breaks into gametes in the form of the translucent bodies.

If Kuckuck (3) is correct in his observation of the union of gametes, there are two interpretations, depending upon whether (1) the gametes must unite to grow, or whether (2) they may grow without union (Oltmanns, 7). If we accept the first of these, the filaments secured in cultivation should be diploid, sporophytic, and the escaping cell contents should be a form of asexual reproduction. But nothing was seen to grow from them; the new plants grow from filaments already there before the escape; and only one chloroplast was found in the germinating cell (zygote?). Sauvageau (10) saw a similar growth in *Leathesia* and concluded that he had zoöspore formation. If we accept the second alternative, the germinating cells (gametes?) which we observed developed without union into a new form of gametophyte, the escaping contents of one filament usually fertilizing certain cells of another. In this case there are two forms of sexual reproduction.

We doubt the observation of union of ciliate cells escaping from the so-called multilocular sporangia, or gametangia. Sauvageau (12) could not find such union either. The small translucent motile bodies formed from the escaping cell contents, we are inclined to believe, may be sperms, but we have not seen them unite with other cells. We believe it probable that a fertilization occurs in cells of the filaments which are about 20 to 25 days old, and that from these grow the new filaments which grow into the well-known large plant.

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XX. GRAPHICAL AND MATHEMATICAL TREATMENTS IN GROWTH STUDIES¹

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Robertson (1908) and Ostwald (1908) simultaneously but independently pointed out the similarity between the curve of the growth reaction and the curve of an autocatalytic monomolecular chemical reaction and suggested that growth under normal conditions is limited by such a chemical reaction. The equation of the autocatalytic monomolecular chemical reaction as derived by Robertson (1908) is

$$\frac{dx}{dt} = kx(A - x)$$

where A represents the ultimate amount of growth attainable and theoretically corresponds to the quantity of growth-producing substance present at the beginning of the growth reaction and exhausted during its course, x is proportional to the amount of this substance converted during any time t , and k is the constant which represents the velocity of the reaction. When integrated, this equation assumes the form,

$$\log \frac{x}{A - x} = kA(t - t_1)$$

which, when plotted, gives an S -shaped curve with a point of inflection at its center. Owing to the flexibility of this curve Robertson (1916, 1923) and Brody (1922) were able to apply it with more or less success to the growth reactions of numerous mammals, including man. Although the growth reactions of most animals and plants are of the sigmoid type, they very seldom if ever are symmetrical and they reach a point of inflection early in the reaction. Hence it was not long until investigators began to point out that the S curve of the autocatalytic monomolecular reaction gave only a rough approximation of the true growth reaction and that the chemical theory embodied in it was not sufficient to explain the normal process of growth. In view of such criticisms Robertson and Brody, as well as Crozier, Pearl and Reed, and others set about to modify the autocatalytic curve so that it would embody the asymmetrical feature of the growth reaction and at the same time provide a chemical interpretation of the growth process.

The first and most natural step in modifying the curve was to add corrective constants to it. Pearl and Reed (1920) seemed to be the most

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successful in this respect, if their success is to be judged by the number of constants they added. The equation of their corrected curve is

$$\frac{dx}{x(A-x)dt} = F(t) = k_1t + k_2t^2 + k_3t^3 + \dots + k_nt^n$$

or, in the integrated form,

$$x = d + \frac{A}{1 + me^{k_1t + k_2t^2 + k_3t^3 + \dots + k_nt^n}}$$

Robertson (1926), on the other hand, not satisfied with the addition only of corrective constants, formulated a new expression by taking the sum of three consecutive autocatalytic monomolecular equations plus a linear equation that he designated as a linear accretion. It is very obvious that such a complicated expression is rather untenable as a chemical interpretation of growth.

Crozier found it necessary to add only one corrective constant to the original equation and he has been the most successful in retaining a rational chemical interpretation. The formula of his corrected equation is

$$\frac{dx}{dt} = (k_1 + k_2x)(A - x)$$

becoming, in integrated form,

$$t = \frac{1}{k_1 + k_2A} \log \frac{A(k_2X + k_1)}{k_1(A - x)}$$

According to Crozier (1926) the velocity constants k_1 and k_2 have the following significance: "The reaction of $A \rightarrow x$ will therefore be governed by the velocity constant k_1 proper to it in the absence of the influence of x , and also by the velocity constant k_2 due to catalysis by x ." Robb (1929) has applied this later equation of Crozier's with a great deal of success to the growth reactions of Flemish and Polish rabbits and their hybrids.

Brody (1927) used a somewhat different method in correcting the original autocatalytic curve. His corrected expression of the growth reaction consists of two equations. The first of these equations applies only to that part of the growth reaction prior to the point of inflection and represents the change in growth as proportional to the growth already made. The formula of this equation is

$$\frac{dx}{dt} = kx$$

The second of these equations applies to the growth reaction following the point of inflection. The formula of this equation is

$$\frac{dx}{dt} = k(A - x)$$

Hence the change in growth following the point of inflection is proportional to the growth yet to be made in order to reach maturity. It is interesting to note that the combination of these two equations results in the original autocatalytic equation, but the interpretation is far from that of an autocatalytic monomolecular chemical reaction.

A growth equation embodying a general biological rather than a chemical interpretation of the growth process, may be derived in the following manner. Minot (1908) showed for a number of animals that the percentage increments in body weight

$$\frac{W_2 - W_1}{W_1}$$

tend to decrease constantly from birth to maturity. These percentage increments may be looked upon as measuring the average growth power of the body cells, if growth power may be defined as the percentage rate of increase in growth. Wright (1926) suggested briefly that the hypothesis that growth power falls off at a constant percentage rate leading to the curve

$$\log \log \frac{c}{w} = a - kt \quad (1)$$

might often give a good fit to growth data. This equation may also be expressed in the form

$$\log W = A - be^{-kt} \quad (2)$$

and

$$L = Be^{-Ce^{-kt}} \quad (3)$$

Equation 1 was fitted successfully by Wright (1926) to growth in weight W of rabbits. Equation 2 was found by Davidson (1928) to give a good fit to growth in weight W of dairy cattle, and Equation 3 was applied by Weymouth with excellent success to growth in length L of the razor clam. The derivation of Equation 2 is as follows:

$$\frac{dw}{Wdt} = P$$

where W equals body weight at any time, t , and P equals growth power of the body cells. Since growth power is assumed to fall off at a constant percentage rate,

$$\frac{dP}{Pdt} = -k$$

by integration,

$$\log P = -kt + C$$

$$P = e^{C-kt} = \frac{dw}{Wdt}$$

by integration,

$$\begin{aligned}\log W &= -\frac{1}{k} e^{C-kt} + A \\ &= A - \frac{e^C}{k} e^{-kt} \\ &= A - b e^{-kt}\end{aligned}$$

In the last equation, A is the logarithm of the weight of the animal at maturity; $100k$ is the constant percentage rate of decrease in growth power according to the interpretation above, and b locates the curve in time; W is the weight at any time t . The equation of the curve for weight W is

$$\begin{aligned}W &= e^{A-be^{-kt}} \\ &= B e^{-be^{-kt}}\end{aligned}\tag{4}$$

where

$$e^A = B$$

This equation is similar to Equation 3 for length L and is S -shaped with the point of inflection at approximately 37 per cent of the final weight. It differs from Brody's growth expression in that it does not have to be divided into two parts in order to make it fit the asymmetry of the growth reaction, and it differs from all of the growth curves mentioned above in that it embodies a general biological rather than a chemical interpretation of the growth process and at the same time requires the utilization of fewer velocity constants.

Brody (1927) has attributed a great deal of biological significance to the point of inflection in the growth reaction. He interprets the point of inflection as corresponding to:

1. The time of maximum velocity of growth
2. Age of puberty in mammals
3. Age of lowest specific mortality
4. Point of reference for the determination of equivalence of age in different animals

Weymouth points out that the biological correspondence interpreted in the point of inflection by Brody is not consistent for all animals, and he is inclined to believe that the point of inflection is not entitled to any particular biological significance. By way of further argument in support of his belief he shows that Equation 4 plotted in the logarithmic form gives a curve, Figure 3, that does not have an inflection and is always convex to the time axis. In other words, he is of the opinion that the theory of growth which is embodied in Equation 4 does not attribute any biological significance to the inflection that occurs in the curves derived from the natural form, Figure 2, of this equation.

GRAPHICAL PRESENTATION OF NORMAL GROWTH

The normal growth reaction may be presented graphically in the form of a velocity curve (see Fig. 1) whose general equation is $\frac{dW}{dt} = f'(t)$, where W equals the body weight at any age t . By integrating the general equation of the velocity curve the equation $W = f(t) + C$ describing the actual weight at any age t may be derived. Figure 2 represents the curve of this general equation. You will notice that the point of inflection on the actual weight curve, Figure 2, corresponds in age to the point of maximum gain in weight on the velocity curve, Figure 1.



FIG. 1

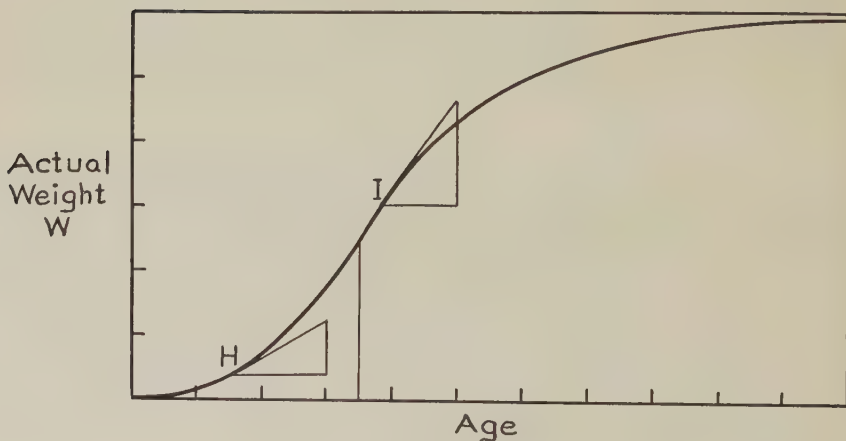


FIG. 2

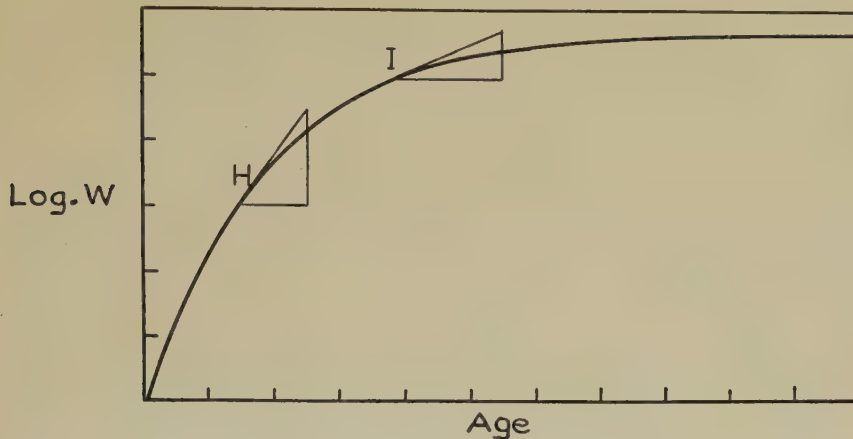


FIG. 3

The normal growth reaction may also be presented graphically in the form of a relative growth curve. In this curve the increase in weight $W_n - W_{n-1}$ is proportional to the weight already attained, i.e., $\frac{W_n - W_{n-1}}{W_n}$ or $\frac{dW}{Wdt}$. The general equation of this curve is $\frac{dW}{Wdt} = F'(t)$, which when integrated gives $\log W = F(t) + C$. Figure 3 represents the curve of this general equation in the integrated form. There are a number of advantages to be gained in describing the course of growth in the form of the relative growth curve. For example, the rates of growth in body weight subsequent to birth of animals differing greatly in initial weight may be satisfactorily compared on this basis, since the increase in weight, $W_n - W_{n-1}$, with age is made proportional to the weight already attained, i.e., $\frac{W_n - W_{n-1}}{W_n}$ or $\frac{dW}{Wdt}$. Again, should one compare the rates of growth in body weight at different ages of the same group of animals as described on the actual weight and relative growth-weight basis, opposing conclusions would be reached. Consider the slopes of the tangents at I and H on the curves in Figure 1 (actual weight) and Figure 2 (relative growth weight). The rate of growth on the curve in Figure 1 is greater at I than at H since the tangent at I is greater than at H. To be sure, the increase in weight $W_n - W_{n-1}$ is greater at I than at H, but when compared to the weight already attained $\frac{W_n - W_{n-1}}{W_n}$ as illustrated on the curve in Figure 3, the rate of growth is less at I than at H. In other words, the rate of growth on the relative growth-weight curve is based upon the change in the physio-

logical activity (reproductive power) of the body cells with increasing age rather than upon the coincident increase in the total number of cells. This seems to be both a reasonable and tenable basis upon which to measure the rate of growth.

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XXI. THE GROWTH OF SALMON¹

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Relatively little study has been made of the growth of the five species of Pacific salmon. This has probably been due, in the first place, to the fact that other problems in the life histories of these fishes have been of more immediate importance. In the second place, satisfactory material has been difficult to obtain. Gilbert (2), working on the red salmon of the Fraser River, was the first to collect any large amount of material. He was able to show that there was a distinct racial variation in the size at maturity, and he also gathered considerable data as to the size and age of the fingerlings as they left fresh water. Frazer (1), working on the salmon of British Columbia, presented data on the size at various ages of all five species of Pacific salmon. All of his material consisted of mature fish which had returned to spawn, and their size at various ages was calculated from the scales. Rich (4), in 1925, made a study of the growth and maturity of Chinook salmon in the ocean. In none of the foregoing was an attempt made to construct growth curves which would show the finer points of growth. Rich, in his paper, pointed out that he was dealing with a population consisting of several different races which obviously had differences in their growth rate.

One of the first difficulties which is encountered in a study of the growth of the salmon is that of obtaining material which is drawn from a single race, or, in the case of a mixture, of getting material in which it is possible to discriminate between the various groups which are present. It has been quite definitely shown in the case of the chinooks of the Columbia and of the red salmon of the Fraser, that the commercial catch is made up of a number of different races. This difficulty can be overcome by the use of material from regions where the catch is restricted to fish from a single small stream. Even in this case the possibility of racial differences is not entirely eliminated, but at any rate it is much simplified.

A second difficulty lies in the fact that it is very hard to secure representative random samples of a given race. The opportunities vary with the different species. In the case of the red salmon, only two chances are usually presented. The first is during the seaward migration of the fingerlings, and the second is when the fish return at maturity. In the case of other species, the opportunities are somewhat increased by the fact that they are taken by troll fishermen; but in most cases of this kind it is impossible to tell from what race or even from what stream these fish are derived. The entire range of ages can usually be found among the migrants

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or the mature fish. However, the fact remains that at any given age, either of migrants or of mature fish, these groups are probably selected and do not by any means represent the size which all the fish of that age have attained.

Material to illustrate this point has been largely drawn from a study of the red salmon of the Karluk River, Alaska, which has been made by Dr. W. H. Rich (3) and the late Dr. C. H. Gilbert.

The growth of the red salmon is naturally divided into two parts, which correspond to the fresh-water and the marine phases of its life. As a result of this division of the normal growth curve into two very distinct portions and the very obvious relation which must exist between the two parts, the study of the growth of these fish presents some very interesting problems which are not found in purely marine or purely fresh-water fishes.

In the case of the salmon, age, as an element in the determination of growth, is usually ascertained from the scales. In many cases the age as determined from the scales has been confirmed by correlation of the catch at varying intervals of time, and occasionally by marking experiments. At Karluk some fourteen hundred marked fish have been recovered after periods of from two to three years in the ocean. In every instance the age as recorded on the scales was in complete agreement with the known time which the fish had spent in the ocean. The number of years spent in fresh water, as determined from scales, is more difficult to confirm, but sufficient evidence has now accumulated to lend a large degree of certainty to these determinations.

The red salmon may leave fresh water at any time after the absorption of the yolk sack, but the great majority migrate down stream in the spring or early summer, which marks the beginning of their second, third, or fourth year. At Karluk a small percentage do not migrate until the beginning of the fifth year. The migration starts at about the same time each year, June 1, and continues until about June 18 or 20. This migration offers almost the only opportunity to obtain samples of fish during their life in fresh water. Numerous attempts in the lake, with various kinds of seines, have yielded but a few hundred fingerlings, and there is strong reason to believe that even if more fish could be secured in the lake, they would not be a random sample of the entire population.

For the early portion of a growth curve, dependence must therefore be placed on samples which are taken during the course of the seaward migration. It might be expected that such samples would yield fairly satisfactory material showing the sizes attained at the end of the first, second, third, and fourth years. In most cases these migrants have made but little growth of the year.

Upon reading the scales of migrants collected in 1926 and 1928, it was found that fish starting their fourth year predominated in the early

days of the migration and that during the latter part of the migration most of the fish were in their third year. The four-year fish averaged 14.5 cm. in length, and the three-year fish averaged 13.5 cm.

In addition, it was found that within each age group there was a decrease in size during the migration. This is shown in Figures 1 and 2. In each case the solid lines show the lengths of male fish and the dotted lines show the lengths of female fish. In each graph the upper pair of lines are for fish in their fourth year and the lower pair are for those starting their third year. Although the decrease in size is not constant throughout the migration, it is apparent that in each case the fish are smaller at the end of the migration than at the beginning. This fact, coupled with the previously mentioned predominance of older and larger fish in the early part of the migration, would indicate that there is some relation between the size of the fish and the time at which they migrate.

There may be several explanations for this, but it is of great importance in growth studies to consider the possibility that any sample which is taken from the migrants can be representative only of the larger fish in any one age group. In all but the oldest group of migrants, there

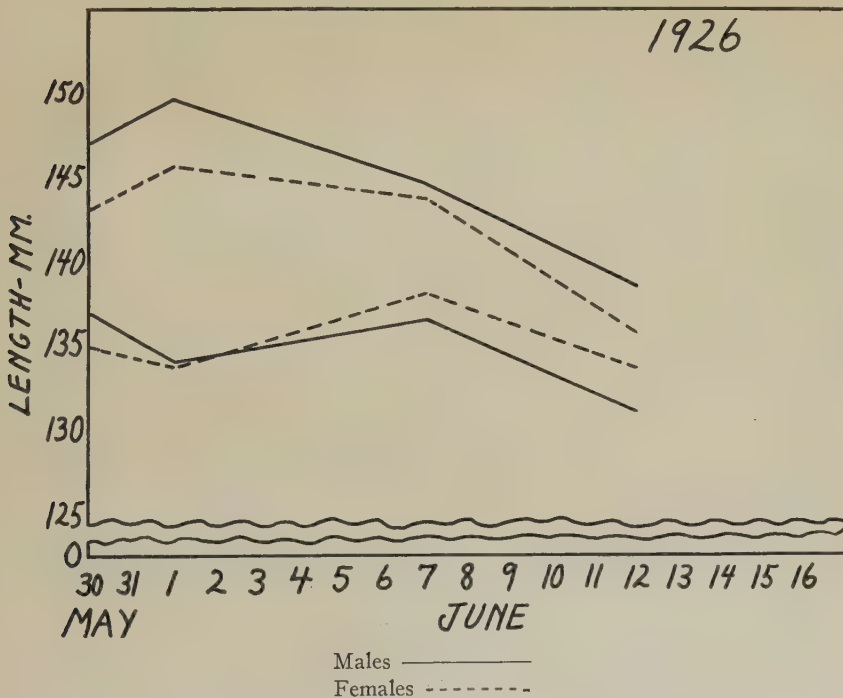


FIG. 1.—Sizes of migrants in 1926. Upper curves, fish in fourth year. Lower curves, fish in third year.

must be fish of the same age remaining in the lake which will not be available for inclusion as part of the growth curve. It would seem quite likely from Figures 1 and 2 that these fish are the smaller ones in their particular age groups; on the other hand, it does not seem likely that the age of migration is entirely dependent upon size. It is obvious, then, that the solid curve of Figure 3, in which the sizes attained at the time of migration are shown, is by no means a complete picture of the growth of Karluk salmon during the first four years of life. It would appear from this curve that the fingerlings grow very little during their fourth year in the lake; but this is by no means a correct inference, since we do not know how large these fish were at the end of their third year, and it is not fair to use the size of the three-year-old migrants as an index.

Similar difficulties are met with in attempting to construct a curve showing the growth while at sea. The red salmon become available only when they reach maturity and return to their native streams. Since a few of them mature after only a little more than one year in the ocean, it would appear possible to construct a curve showing the sizes at all ages. Yet this is, of course, not the case, since maturity itself is a form of

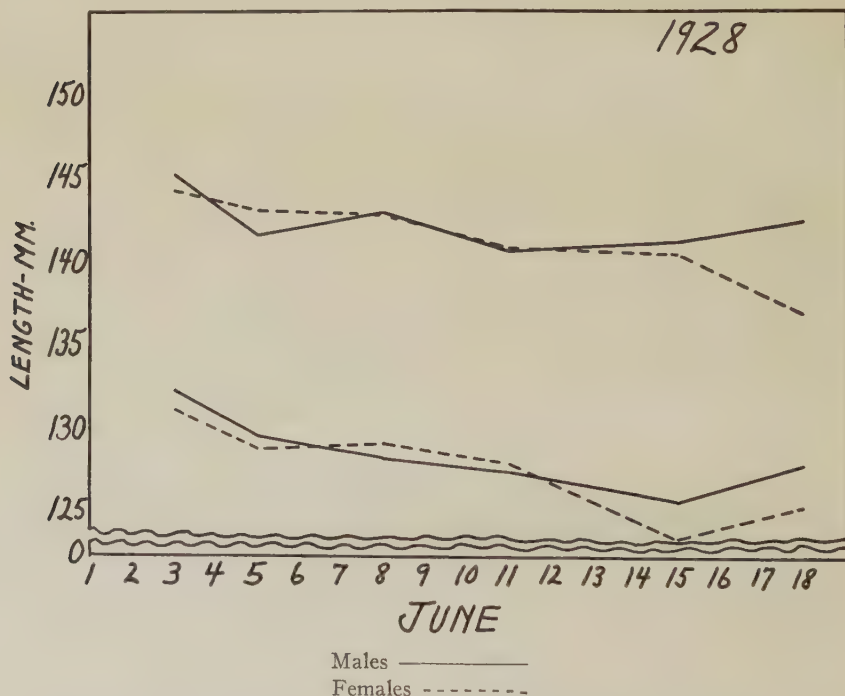


FIG. 2.—Sizes of migrants in 1928. Upper curves, fish in fourth year. Lower curves, fish in third year.

selection and, except in the case of the oldest group of fish, there are always others of the same age which have not yet reached maturity and which remain at sea for one or more years of growth.

Even individual age groups do not appear to be homogeneous from the standpoint of growth. Figure 4 (p. 258) shows the average lengths of the males and females of the 5₃ age group (fish which left fresh water early in their third year and matured in their fifth year) in the run of 1926. If this was a homogeneous group, one would expect to find a steady increase in size during the course of the run, which lasts from June to September. Instead, it is found that these fish grow progressively smaller during June and then increase rapidly in size until August. From August to the end of the season, there is again a slight decrease in size.

From the sizes at the time of migration and maturity we can gain some idea as to the growth rate in fresh and in salt water. This is shown in Figure 3. The solid curve represents the different sizes attained during from one to four years in the lake. The dotted curves indicate the size reached after periods ranging from two to four years in the ocean. The great difference is particularly well shown by the group which migrated at three years of age and which had an average length of 14.5 cm., and the group which migrated as fry during their first year and matured at three

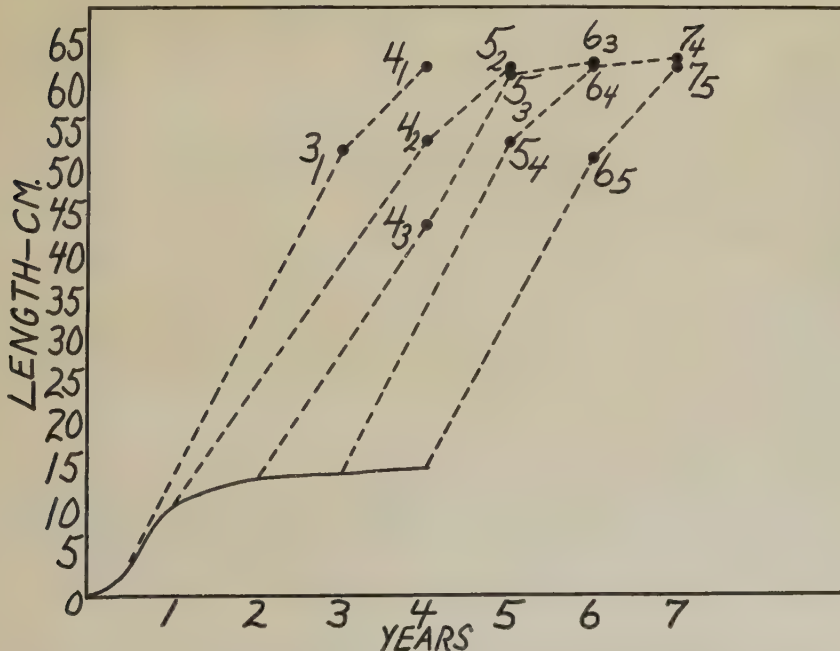


FIG. 3.—Sizes of Karluk red salmon at the time of migration and at maturity.

years of age. These fish, which had spent practically the entire three years in the ocean, had an average length of 53 cm.

There appears to be little relation between the time spent in either environment and the age at maturity, but there do appear to be two sizes of maturity, one at about 54 cm. and the other at about 63 cm. With two exceptions, this latter group has spent an additional year in the ocean. The 6_3 's and the 7_4 's are the two exceptions. These fish spent two additional years in the ocean but made little gain in size during the second year. In making comparisons of this kind, we are again in error, since we know nothing about the size of the 6_3 's or 7_4 's at the end of the previous year.

It is quite apparent that there is a well-defined maximum size which is reached by some fish during their fourth year and by others during their seventh.

There is probably only one method by which an adequate growth curve could be constructed for the red salmon. This is through calculations made from the scales. This method has been used with more or less success for a number of different fish and is based on the fact that the scale, in general,

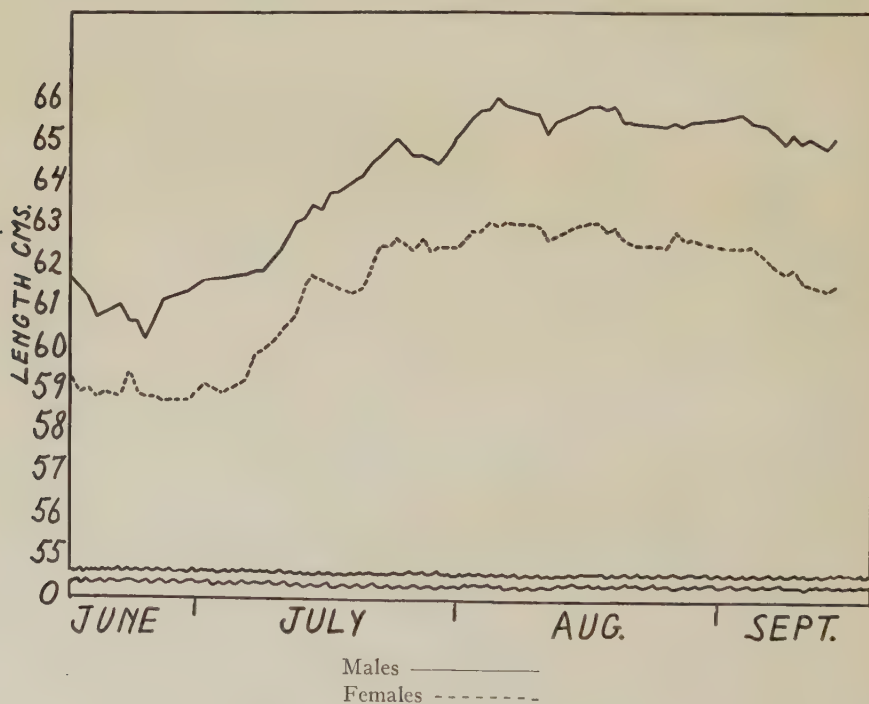


FIG. 4.—Average lengths of males and females of the 5_3 age-group in Karluk red-salmon run of 1926.

grows as the fish does and that, if each year's growth of the scale is sufficiently well defined, it is possible to calculate the length of the fish from these year marks. A difficulty, however, arises in such use of the scales, since a number of workers have found that there is not a straight-line relation between scale growth and fish growth. The exact character of this relation can probably be established for the red salmon, and when this is done it will be possible to follow the growth of each age group through its growth in both fresh and salt water. Such a study would have great value both from the standpoint of racial characters and from that of age at maturity.

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XXII. THE EXISTENCE AND CAUSES OF DOMINANT YEAR CLASSES IN THE ALASKA HERRING¹

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In this investigation we have attempted to discover how the species is faring under the changed conditions of survival incident to an intensive fishery. In doing this, not only must we show what changes in abundance have occurred, but we must show what part of these changes may be due to natural causes. In order to do this a knowledge of the size and age composition of the catch is fundamental. In many species of fishes, owing to variations in hydrographic or other conditions, the amount of success attending spawning varies greatly from year to year, it being quite normal to have periods of years when but few young survive and occasional years in which exceptionally large numbers of young survive. These unusually abundant year-classes, thence termed "dominant" year-classes, have been found in the cod, the plaice, the mackerel, and in the very close relations of the Pacific herring, the California sardine and the Atlantic herring; and their presence causes natural fluctuations in abundance. In proving the existence or non-existence of this phenomenon, samples are needed over a period of several years. In this investigation such a series is available in the vicinity of Latouche, Prince William Sound.

In order to have comparable data for the six years under consideration only the samples taken in June and July have been included. The greater portion of the annual catch is taken during these months. The body lengths shown in Figure 1 were first grouped in half-centimeter categories and then smoothed twice by threes to remove minor modes due to chance sampling. In examination these smoothed-percentage length distributions reveal a shifting of sizes from year to year. In 1924 there are two modes, *A* and *B*; in 1925 the two are still discernible; but in 1926 they have merged, probably owing to mode *B* approaching closer to mode *A*, as the distance between the modes is bound to become less as the fish grow older, due to the decreasing rate of growth.

In 1927 a trough appears at *C* which is seemingly due to a year-class less numerous than *A* (or *A* and *B*). Below *C* is a pronounced mode at *D* which also shows as a small mode in 1926. In comparing these curves one must remember that the samples in question are taken from the commercial catch, implying that, aside from the question of our samples representing the catch, there is the question of the catch representing the population. The gear used, in this case purse seines of one and one-half inch mesh, is selective in its action, owing partly to the smaller fish escaping,

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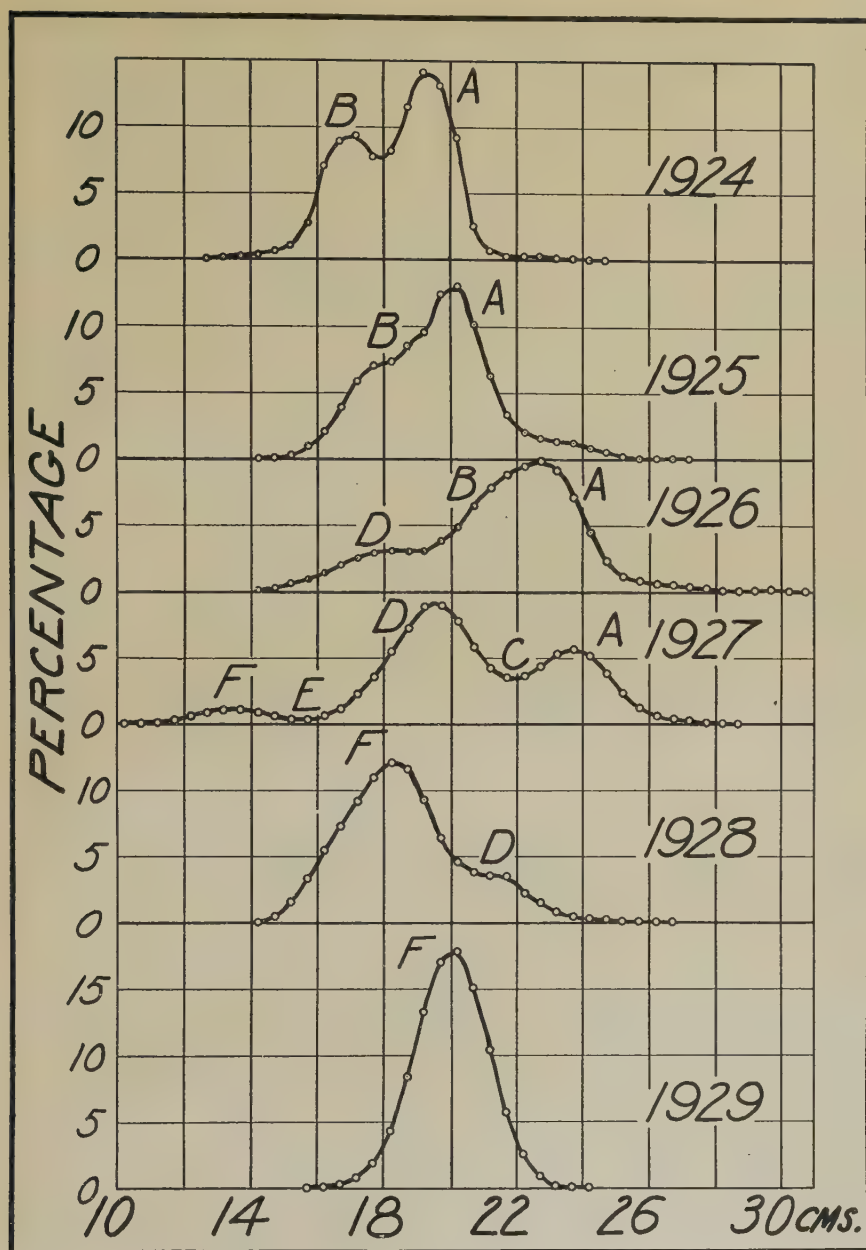


FIG. 1.—Body length frequencies from the vicinity of Latouche, Prince William Sound, shown as percentages of each distribution (smoothed twice by threes).

partly to the selection of certain schools by the fishermen, and partly to the fish themselves, which school according to size and sexual maturity. Owing to this selective schooling and selective fishing, herring below a certain size are poorly represented. However, in 1927, a mode appears at *F*, the only fish of such small size to appear in our samples. This appearance may be due to one or both of two causes: to the unusual abundance of this year-class, or to the individuals growing faster than usual so that the larger of the year-class schooled with the older fish. This same group is overwhelmingly dominant in 1928 and 1929.

Although in this form the data show well the dominance and progression of certain size-groups, yet they do not show the relative lack of certain size-groups, not only as compared to the other sizes for the same year but as compared to the average of the same sizes over the entire period of six years. The average curve for these six years (Fig. 2, top curve) was obtained by summing the weighted frequencies (percentage frequencies) of each of the six annual curves and dividing by six to get the arithmetic mean for each ordinate. With this average curve as a base, the deviations of each of the six years from the average curve were plotted so that a frequency greater than the average appears above the line (as solid black), less than the average below the line. The progression of size-modes in these curves is too obvious to require much discussion. The more dominant modes of Figure 1 as *A*, *B*, and *F*, show very clearly and unmistakably (in Fig. 2) the progression of size-groups.

That this progression of sizes is due to the growth of fish of dominant age groups is sustained by comparing age-analyses (Fig. 3, p. 265). While there is undoubtedly a small percentage of error in the age-readings, yet they are of great value in interpreting the significance of the size-modes, and the consistency of the results obtained by the two methods is further evidence of their validity.

Having demonstrated the existence of dominant year-classes, certain questions arise: "What is the cause of this phenomenon and with what factors is it related? Must we wait until the herring of any particular year-class are adequately represented in the catch three years after spawning before we can judge of the success or failure of that spawning?"

In Prince William Sound the herring spawn in April and May, attaching their eggs to seaweeds and kelps along the shore, chiefly between tide lines, where they are exposed to the air twice daily, owing to the rise and fall of the tide. The eggs, which are only slightly over a millimeter in diameter, hatch in two or three weeks, and the tiny larvae, about 6 to 8 mm. in length, are very frail.

Assuming the most critical period in the life of the herring to be somewhere between the deposition of the eggs and the first few weeks of larval life, we decided to make an attempt to correlate the success of

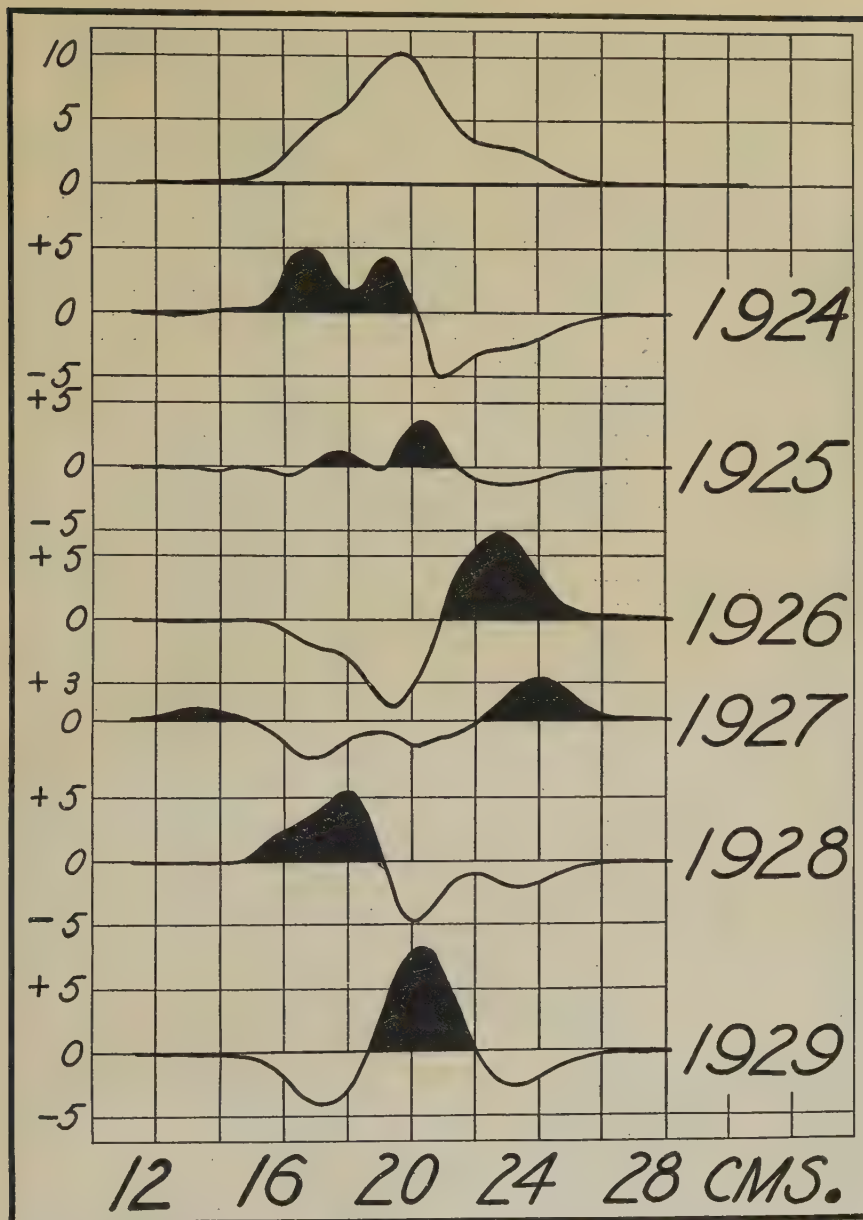


FIG. 2.—Showing the percentage deviations of each of the annual length-frequency curves (of Fig. 1) from the six-year average (top curve).

TABLE 1
PERCENTAGE LENGTH FREQUENCIES FOR JUNE AND JULY FROM THE VICINITY
OF LATOUCHE SMOOTHED TWICE BY THREES

Body Lengths (Millimeters)	Percentage						Average Per- centage
	1924	1925	1926	1927	1928	1929	
90-94				.01			
95-99				.03			
100-104				.04			.01
105-109				.05			.01
110-114				.11			.02
115-119				.32			.05
120-124				.61		.01	.10
125-129	.03			.92		.01	.16
130-134	.16			1.09		.02	.21
135-139	.32		.02	1.09	.02	.01	.24
140-144	.46	.02	.10	.93	.11	.01	.27
145-149	.66	.19	.27	.59	.56		.38
150-154	1.15	.36	.58	.38	1.64	.01	.69
155-159	2.81	1.00	.97	.30	3.43	.05	1.43
160-164	7.05	2.06	1.50	.64	5.49	.14	2.82
165-169	8.99	3.96	2.03	1.21	7.41	.36	4.00
170-174	9.46	5.87	2.60	2.29	9.23	.79	5.05
175-179	7.84	7.03	3.02	3.62	11.02	1.95	5.76
180-184	8.22	7.46	3.16	5.50	12.19	4.42	6.84
185-189	11.55	8.51	3.21	7.36	11.63	8.48	8.47
190-194	14.20	9.67	3.22	8.86	9.29	13.34	9.78
195-199	13.13	12.46	3.85	9.03	6.49	17.04	10.35
200-204	9.21	13.07	4.98	7.84	4.63	17.86	9.62
205-209	2.58	10.20	6.60	5.93	3.94	15.18	7.42
210-214	.69	6.30	7.91	4.26	3.58	10.48	5.55
215-219	.37	3.41	8.92	3.51	3.11	5.77	4.19
220-224	.35	2.05	9.57	3.68	2.31	2.56	3.43
225-229	.29	1.65	9.99	4.45	1.54	.95	3.15
230-234	.21	1.43	9.21	5.30	.87	.33	2.90
235-239	.14	1.26	7.24	5.66	.51	.12	2.49
240-244	.06	.91	4.52	5.17	.33	.04	1.84
245-249	.03	.55	2.39	3.86	.27	.01	1.19
250-254	.01	.23	1.24	2.32	.18	.01	.67
255-259	.01	.15	.83	1.23	.14	.01	.40
260-264		.15	.67	.66	.09	.02	.27
265-269		.11	.52	.45	.04	.01	.19
270-274		.06	.39	.32		.01	.13
275-279		.01	.29	.21			.09
280-284		.01	.16	.11			.05
285-289			.06	.04			.02
290-294			.11				.02
295-299			.22				.04
300-304			.33				.05
305-309			.22				.04
310-314			.11				.02
Number of specimens.....	2,000	6,889	1,041	785	615	1,585	12,915
Number of samples.....	10	70	17	14	14	20	145

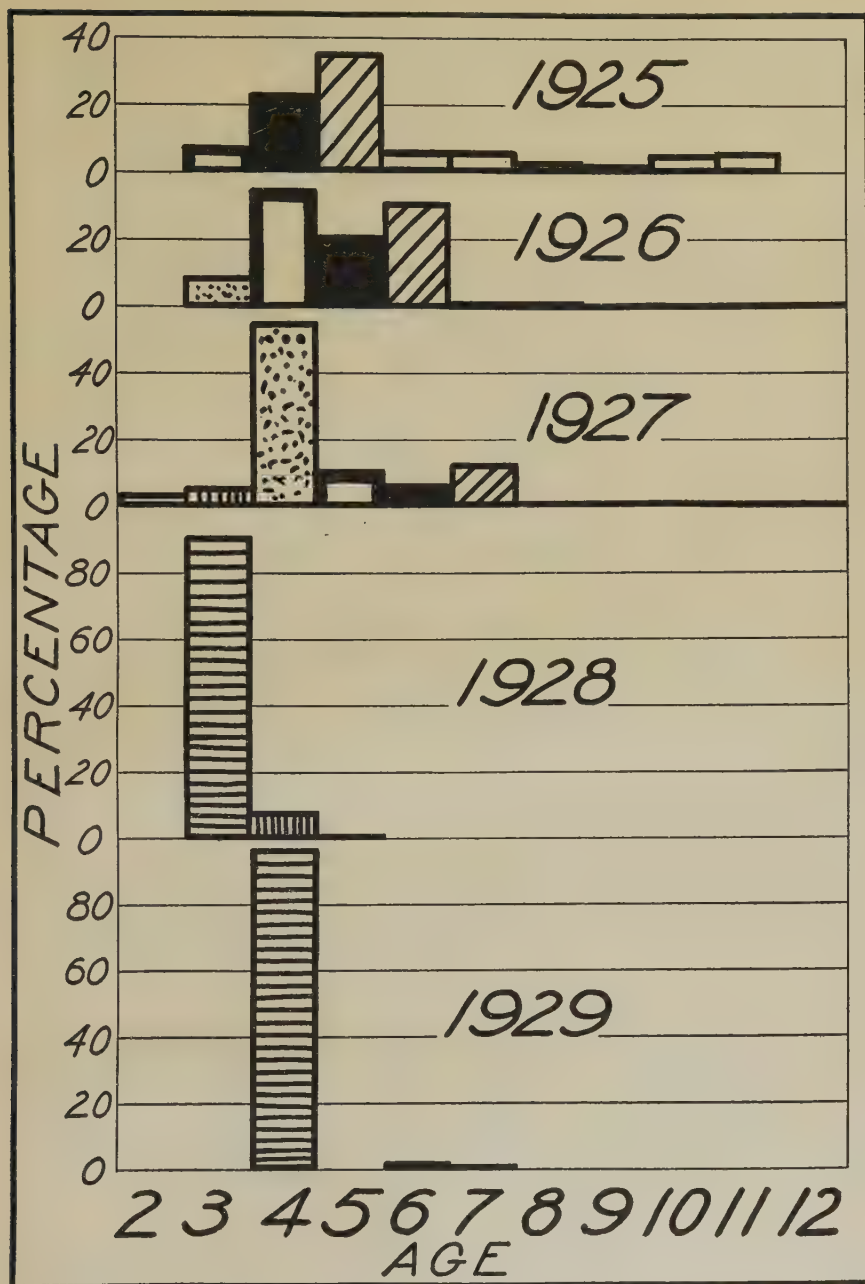


FIG. 3.—Age frequencies from the vicinity of Latouche, Prince William Sound, shown as percentages of each distribution.

TABLE 2
PERCENTAGE AGE DISTRIBUTION FROM THE VICINITY OF LATOUCHE

Age	1925		1926		1927		1928*		1929*	
	Actual	Per-centage	Actual	Per-centage	Actual	Per-centage	Actual	Per-centage	Actual	Per-centage
2.....	...	...	...	...	25	4.0	...	...	...	...
3.....	13	7.9	35	9.0	38	6.1	80	91.0	...	...
4.....	42	25.5	137	35.4	43	55.2	7	8.0	97	97.0
5.....	59	35.8	82	21.2	69	11.1	1	1.0	...	...
6.....	11	6.7	120	31.0	43	6.9	...	...	2	2.0
7.....	11	6.7	3	.8	82	13.2	...	...	1	1.0
8.....	6	3.6	3	.8	2	.3	...	...	...	...
9.....	4	2.4	1	.3	9	1.4	...	...	...	...
10.....	9	5.5	1	.3	5	.8	...	...	...	...
11.....	10	6.1	3	.8	4	.6	...	...	...	...
12.....	...	...	2	.5	1	.2	...	...	...	...
Number ..	165	...	387	...	621	...	88*	...	100*	...

* Only a fraction of the ages for these years have been read to date.

spawning, as shown by the relative importance of the year-classes in the catch, with the physical conditions during this period. The only data available on the physical conditions were the *Climatological Data* published by the United States Weather Bureau. In examining these it was obvious that the success of spawning did not show a very high correlation with the maximum or minimum monthly temperatures or with the mean annual temperatures. The highest correlation is with the averages of the mean monthly temperatures of March, April, May, and June, taken as a whole, a period extending from a month or more previous to spawning to a month or six weeks after the hatching of the eggs. The mean annual temperatures for this period of four months are shown in Figure 4 for Seward, Cordova, and Latouche, all in the general vicinity of the Prince William Sound spawning grounds.

In correlating temperatures with the year-classes we encountered difficulties, for, although a year class may be more or less abundant, yet, having no accurate measure of its actual abundance, it cannot be given a numerical value. For this reason in correlating their abundance with the temperature it was necessary to use Spearman's rank coefficient of correlation

$$r = 1 - \frac{6\sum D^2}{N(N^2 - 1)}$$

in which D is the difference in the corresponding ranks in two related series and N is the number of cases.

Using this method and ranking the year-classes from 1920 to 1927 by

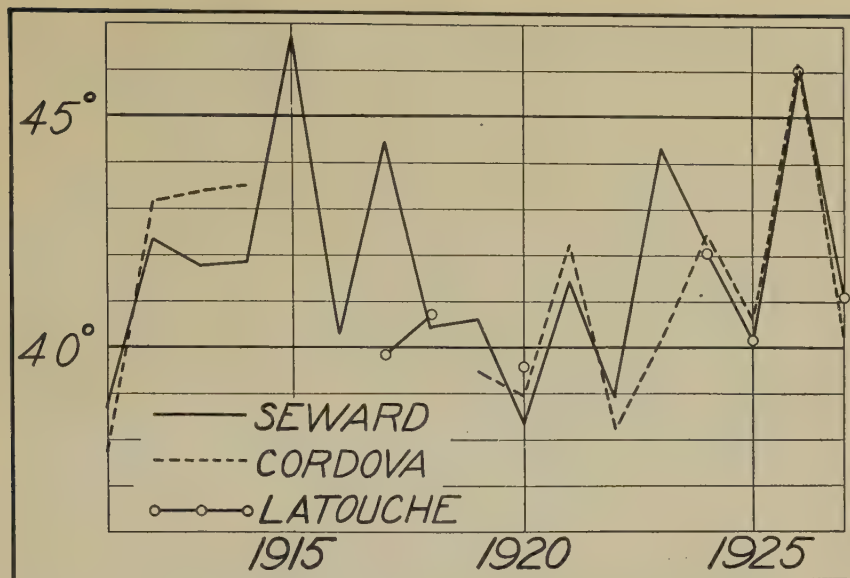


FIG. 4.—Mean annual temperatures for the combined months of March, April, May, and June, for Seward, Cordova, and Latouche, Alaska.

TABLE 3

MEAN ANNUAL TEMPERATURES FOR THE COMBINED MONTHS OF MARCH, APRIL, MAY, AND JUNE FROM SEWARD, CORDOVA, AND LATOUCHE

Year	Seward	Cordova	Latouche
1908.....	41.40		
1909.....	39.88		
1910.....	38.43	40.55	
1911.....	38.80	37.70	
1912.....	42.35	43.15	
1913.....	41.73	43.38	
1914.....	41.88	43.50	
1915.....	46.70		
1916.....	40.30		
1917.....	44.25		39.83
1918.....	40.45		40.07
1919.....	40.63	39.50	
1920.....	38.35	38.95	39.63
1921.....	41.43	42.23	
1922.....	38.98	38.18	
1923.....	44.33	40.13	
1924.....	42.23	42.43	42.10
1925.....		40.53	40.15
1926.....		46.23	46.18
1927.....		40.20	41.05

their relative importance as shown in Figure 3, we obtain a ρ of .71, which converted into terms of r , the coefficient of correlation, gives .73, with a probable error of $\pm .11$. This is a surprisingly high coefficient of correlation, and before accepting it we must consider one important aspect, namely, the ranking of the year-classes.

Returning to Figure 3 it is quite obvious that the 1926 year-class (horizontally barred) is entitled to highest rank. Figure 3 shows that the 1921 year-class (diagonally barred), which shifts from five years in 1925 to seven years in 1927, is entitled to second place. Third, we ranked the 1924 class (stippled), which is dominant in 1927 at four years of age over the 5- and 6-year-olds. It is not fair to compare this class with the others in 1926, since, as a rule, they are not adequately represented in the catch until they are four years of age. The 1923 year-class (with heavy border), four years of age in 1926 and five in 1927, obviously comes next, and after that the 1922 year-class (solid black), which is but a trifle less abundant. That leaves but three more year-classes, 1920, 1925, and 1927, to rank, as the data beyond the 1920 year-class are not sufficient to warrant any conclusions. Of these three the 1927 year-class, which does not appear as 3-year-olds in 1929, was ranked at the very bottom. There remain only 1925 and 1920. The 1925 year-class (vertical bars) shows about the usual number of 3-year-olds in 1927 and is abundant enough to show a few 4-year-olds in 1928 in spite of the overwhelming dominance of the 3-year-olds; therefore, 1925 was ranked above 1920, which is almost totally lacking in both 1926 and 1927. However, if the ranking of these two year-classes were changed, the correlation would scarcely be affected. Indeed, five different rankings of the year-classes gave coefficients of correlation ranging only from .66 to .78 (Table 4).

TABLE 4
RANKINGS OF MEAN TEMPERATURES AND DOMINANT YEAR-CLASSES

Rank	Ranking of Temperature	Ranking of Year-Classes*				
		1	2	3	4	5
1.....	1926	1926	1926	1926	1926	1921
2.....	1924	1921	1921	1924	1921	1926
3.....	1923	1924	1924	1921	1923	1924
4.....	1921	1923	1923	1923	1924	1923
5.....	1927	1922	1922	1922	1922	1922
6.....	1925	1925	1920	1925	1925	1925
7.....	1920	1920	1925	1920	1920	1920
8.....	1922	1927	1927	1927	1927	1927
Coefficient of correlation..		.73	.71	.78	.71	.66
Probable error.....		.11	.12	.09	.12	.13

* The most probable ranking is that on the left, the next one is to the right, and so forth.

Some objections may be raised at this point, because, in judging the relative success of spawning, we have not made any allowances for the differences in numbers of spawners present on the spawning grounds in different years. To justify our disregard of this factor we give Table 5, in which we have compared the ranking of the year-classes with that of criteria of the number of spawners present each of the brood years under consideration. The herring spawn in April and May, and the catch is made, *after spawning*, from June to October but largely in the last part of June and in July. The total catch is not as a rule a good criterion of abundance, but since one unacquainted with the fishery might argue (as is occasionally the case with other fisheries) that competition between units of fishing effort might reduce the catch per unit effort (in this case a purse seine boat), we have attempted correlations with both the total catch and the catch per unit of gear. We obtained an r of $-.10 \pm .24$ and $-.09 \pm .24$, respectively, obviously a correlation of no significance whatsoever.

TABLE 5

RANKINGS OF YEAR-CLASSES AND OF FACTORS USED AS CRITERIA OF THE ABUNDANCE OF SPAWNERS IN THEIR BROOD YEAR

Rank	Ranking of Year-Classes	Rankings of Factors Used as Criteria of the Abundance of Spawners in the Brood Year of the Various Year-Classes		
		Total Catch of Brood Year	Catch per Boat of Brood Year	Portion of Catch per Boat Pickled in Brood Year
1.....	1926	1922	1922	1922
2.....	1921	1923	1920	1921
3.....	1924	1925	1921	1920
4.....	1923	1920	1923	1923
5.....	1922	1921	1925	1925
6.....	1925	1924	1924	1927
7.....	1920	1926	1926	1924
8.....	1927	1927	1927	1926
Coefficient of correlation.....		— .10	— .09	— .24
Probable error.....		.24	.24	.22

Since a small fraction of the catch is immature, we have also attempted a correlation with the catch per boat that was used for pickling (Table 5), which consists only of the larger and therefore indubitably mature individuals. The result, an r of $-.24 \pm .22$, also shows a lack of any correlation with the success of spawning.

It seems logical to assume that the air temperatures show their high degree of correlation with the success of spawning because they are closely

correlated with the surface-water temperatures. That such a close correlation exists is shown by Figure 5, which shows air temperatures and surface-water temperatures, averaged by five-day periods, which were

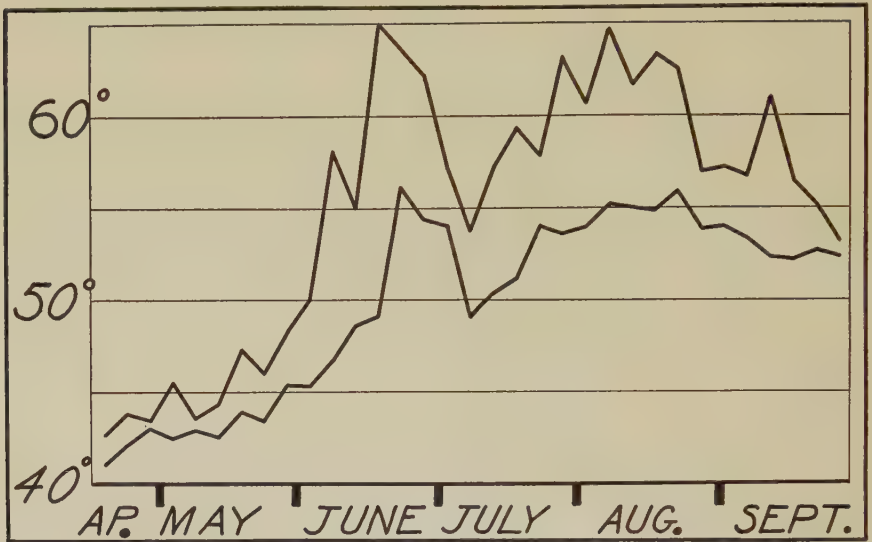


FIG. 5.—Showing correlation between air temperatures (upper) and surface-water temperatures (lower) taken daily at 10:00 A.M. from a Bureau of Fisheries vessel patrolling Kodiak Island in 1926.

taken daily at 10:00 A.M. from a Bureau of Fisheries vessel patrolling Kodiak Island in 1926.

Why such a correlation exists is at present a matter of conjecture. The fact that the correlation is not over a very limited period of time but for four months suggests that it is not due to any direct effect upon the eggs. It is more likely to be associated with the food supply since the larvae at the time of the absorption of the yolk are able to take only certain types of planktonic food, and if such were not available in sufficient quantity it is conceivable that great numbers would perish.

On the other hand, it may not be a question of food supply, but one of enemies. Medusae, ctenophores, sagittae, etc., if present in numbers, might easily destroy enormous quantities of the larvae.

The knowledge that such a high correlation exists between air temperature (or water temperature) and the success of spawning may prove to be of great importance from the standpoint of conservation, enabling the forecasting of the increments of young fish that are to be added annually to the population.

XXIII. THE INTERNAL CAUSES OF GROWTH AND DIFFERENTIATION IN PLANTS

BY H. L. VAN DE SANDE BAKHUYZEN

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If we compare the growth and other life-processes of plants with those of animals, the most striking difference is the fact that animals represent more closed systems, whereas plants are more open systems. Whereas animals carry their own constant environment with them, plants do not. The internal environment of plants is largely dependent upon the external environment, as temperature, light, and available water. In this way the effect of external conditions upon the integral life-processes in plants must be much more important than it is in animals. As life-processes are ultimately bound up with the living protoplasm, we must study how the environment acts upon the colloidal properties of the protoplasm. But it is not sufficient to speak about the protoplasm as such, as this living substance is not a static thing but a dynamic one, i.e., it changes with time and age.

It has been suggested by several botanists that growth is a hydration process of the protoplasm. Now the term "hydration" is often used in a rather loose sense; therefore we must try to give a more exact definition of this term. In botany we make a distinction between the degree of turgescence or actual osmotic pressure and osmotic pressure maximum or potential osmotic pressure. Exactly the same difference we must make between degree of imbibition or hydration and the imbibition maximum or imbibition capacity or hydration capacity. Suppose we have a lump of gelatangel; the weight of the gelatin, i.e., the amount of water taken in per gram of gelatin, depends upon the relative vapor pressure of the surrounding air with which it is in equilibrium. The relative vapor pressure determines the degree of saturation of the gelatin. Now we place our gelatin in distilled water or in a molar solution of KI. The gelatin will swell until the swelling force is in equilibrium with the cohesion of the gelatin particles. But in distilled water its imbibition maximum or water-holding capacity is much lower than in KI, in which the gelatin swells infinitely to form a sol. Now we have changed the potential swelling or hydration capacity. I do not need to remind you that the hydration capacity of an ampholyte is minimum at the isoelectric point and increases as we move away from this point. At the same time the electric charge of the particles increases.

We have to make the same distinction if we study the hydration of the protoplasm. Plants show continuously a water deficit in their organs. Animals do not. The reason of this, as you know, is that by day the water

loss by transpiration exceeds the water intake by the roots. This water deficit in the plant organs involves a water deficit in the protoplasm, i.e., the degree of water saturation of the protoplasm depends upon external conditions.

On the other hand, I mentioned that several investigators call growth a hydration process. By this is meant an increase in hydration capacity or swelling maximum.

I will show now that not only is the degree of saturation of the protoplasm important for growth-processes but also that we have to assume that the water-holding capacity of the protoplasm changes with age.

With Sachs (16) we assume three periods of growth: the meristematic or embryonic period; the period of cell elongation; and the period of differentiation.

In the meristematic organs, the growing points of stems and roots and in the cambium, which forms the secondary wood and bast, the cells have a very small size and are filled with protoplasm without vacuoles and with a very large nucleus. Pearsall and Priestley (13) have advanced a very suggestive theory which assumes that the meristematic plasma has a very low electric charge and low hydration capacity, indicating an approach to the isoelectric point. Indeed it has been found that the imbibition capacity of such cells is much lower than that of full-grown cells, also that the osmotic suction is low, lower than that of any other tissues (14). As little free water is present in those embryonic cells, according to the mass law those chemical processes will be favored which release water, i.e., the condensation processes, such as synthesis of higher proteins and protoplasm, will take place (13).

As cell divisions are going on, it is practically certain that variations of electric charge¹ and hydration occur in the protoplasm of the meristematic cells, but it is as yet premature to state anything more definite about the magnitude or the directions of those changes. But I want to emphasize one thing, and that is that we have to make a distinction between the peripheral protoplasm and the interior or perinuclear protoplasm. Several investigators (2, 5, 12) have emphasized such a difference and this agrees with the fact that the isoelectric point of the protoplasm is at a pH of about 4.2–4.3, whereas that of the nucleus is much higher, 5.0–5.5 (10). This means that the protoplasm is farther away from the isoelectric point than the nucleus, i.e., it is more negatively charged¹ than the nucleus. The protoplasm around the nucleus, however, is more or less under the influence of the nucleus and therefore is less negatively charged than the peripheral protoplasm. Indeed it has been found by Mommsen (8) that in lymphocytes the perinuclear plasma had its isoelectric point at a pH

¹ By the term "charge" is meant the ξ - or electrokinetic potential, not the ϵ - or thermodynamic potential.

of 5.25, whereas the peripheral plasma had its isoelectric point at a pH of 4.5; i.e., the peripheral plasma was more negatively charged than the perinuclear part. In the meristematic cells the perinuclear protoplasm has enough negative charge, however, to retain its colloidal stability.

Now, as the cell grows older, vacuolation begins, and at first the nucleus is suspended in the midst of the sap spaces, but later these spaces fuse together to form one large vacuole and the nucleus and protoplasm are found peripherally lying against the cell wall. As I stated already, many biologists think that as the cell grows, hydration of the protoplasm increases. Consequently many investigators assume that vacuolation is due to an increase of hydration of the plasma. Others, however, believe that the reason of vacuolation is just the reverse, i.e., the result of a dehydration of the plasma. I think that the two views are not incompatible and that as the cell grows the hydration capacity of the external protoplasm increases, whereas that of the internal protoplasm decreases to such an extent as to lose its colloidal state, so that it gives off its colloidal hydration water and absorbed solutes and forms a sap space filled with free water and the solutes. This is the vacuole, the result of a demixing or salting-out of the internal protoplasm. This reconciliation of opposite points of view is substantiated by the fact that the internal protoplasm is in contact with the vacuole, which is acid and has a pH of about 5.0 or 5.5. If the internal protoplasm has its isoelectric point at a pH of 5.0–5.5, it is at its isoelectric point or is even positively charged.

In this way we come to a picture in which the negative electric charge of the peripheral protoplasm increases as the cell grows, whereas the electric charge of the interior of the protoplasm becomes relatively positive and loses water to the vacuole. As the cell grows, the difference between both charges increases up to the time that the cell begins to age. After that the difference decreases, ageing sets in, and finally the cell dies. This difference in charge at both sides of the protoplasm forms an asymmetric system which is rather comparable with the protein-chain set up by Matsuo (6) and Mond (7). This must yield an electromotive force. Indeed it has been found by Osterhout and collaborators (12) that there is a potential difference between the inside and the outside of a cell.

If we decrease the difference of charge between inside and outside, water and solutes must be lost; whereas, if this difference is increased, water and solutes must be taken in and accumulated in the vacuole. This is the *colloid-chemical basis of permeability*. We can change this difference by changing the negative charge of the outside of the protoplasm by adding some lyotropic salts, acids, bases, or organic substances. I was able to prove that even if the negative charge of the outside is destroyed by placing the cell at a pH of 4.2–4.3, the isoelectric point of the peripheral protoplasm, we can stabilize the protoplasm by the addition of 1/40 N urea or

small traces of alcohol (unpublished data). I want to emphasize here again that the electric charge of the protoplasm depends not only upon the H-ion concentration but is also affected by neutral salts and organic substances.

Also the exudation processes and root pressure may be explained in this way, by assuming a unilateral change in charge of the external protoplasm. *The internal environment of the protoplasm is largely independent of the external environment*, as the pH of the vacuole is not affected by external pH of the medium (4).

As I have stated, the difference between the internal and the external protoplasm increases as the cell grows older and as more water and solutes are taken in. There is, however, another reason why more water is taken into a growing cell, and that is the decrease of the elastic pressure of the cell wall. This decrease in elasticity, or increase in plasticity of the cell wall, means that less force is opposed to the osmotic force exerted by the solutes of the vacuole. This is the second reason of cell extension. As gradually in the cell wall new particles are inserted between the old ones, fixation occurs, and the reversible extension of the cell wall becomes an irreversible one. Also this increase in plasticity or decrease in elastic modulus of the cell wall is probably due to an excessive increase in hydration capacity, as Northrup and Kunitz (11) found for the effect of lyotropic salts on gelatin.

Now we must explain the reason for this increase of negative charge in the peripheral protoplasm and the decrease of elastic modulus in the cell wall. Went (21), in his excellent paper on growth-promoting substances, found that the meristematic cells secrete substances which diffuse into the growing region and which are indispensable for growth. Without growth hormones, no growth. This fact is substantiated by many authors, and Priestley's objections (15) have been refuted by Söding (17). Went advances the theory that these substances decrease the elastic modulus of the cell wall of the growing cells, thereby enabling the vacuole and the cell to take up more water and to increase in volume. Considerations to discuss which would lead me too far here make it necessary to assume that these substances also increase the negative charge of the outer protoplasm, thereby increasing the difference between inside and outside and increasing the amount of water and lyotropic salts taken up. We can explain therefore both internal causes of growth—the increase of hydration of the protoplasm and the increase of hydration of the cell wall—as colloidal effects of the action of the growth-hormones.

The amount of growth-hormones present determines directly the magnitude of plastic extension of the cell wall and the hydration of the plasmatic colloids. The more growth-hormones are formed, the greater their effect. This is, for instance, the case in etiolated plants, which grow

fast and have a very high water-content in their organs. If the plant is illuminated, fewer growth-hormones are formed and the water-holding capacity and volume of the cells is much smaller, i.e., it grows much more slowly, and its final length is less. In this way we can explain the effect of light upon growth in a colloid-chemical way.

Also the amount of water available to the plant is important for the protoplasm and also for growth. If less water is present, less water is taken into the cells, and the volume of the vacuole and of the cells is less, the osmotic concentration of the cell sap is high, and the water content of the protoplasm is low. Consequently growth is retarded. But the water-content of the protoplasm may become so low as to injure the life-processes of the protoplasm. This finally causes death. Walter (18, 19, 20) found that each species shows a certain limit to which the protoplasm can lose water but below which limit the cell dies. If, therefore, a plant is transferred to an environment in which so little water is available to the plant that this limit is exceeded, the plant cannot grow or survive. This is the *colloid-chemical basis for ecological behavior of plants*.

But before this limit is reached, the amount of water present in the plant and also in the meristematic cells varies. Now we must assume that the type of cell formed by the meristematic cells depends upon the water-content of their protoplasm. If the amount of water is high in the protoplasm, be it because the degree of saturation is high or because its hydration capacity is high, juicy, long-living cells, such as parenchyma, collenchyma, etc., are formed, if the water-content is low, the cells gradually die, and dead or woody tissues, such as vessels, tracheids, fibers, sclerenchyma, etc., will be formed. All kinds of xeromorphic structures such as sun and shade leaves (19), succulence, and xerophytic structures proper can be explained as a result of low hydration of the protoplasm of the meristematic cells. This is the *colloid-chemical basis of differentiation and adaptation*.

Pearsall and Priestley (13) assumed that the cambium was situated on a gradient of increasing acidity, from the phloem over the cambium to the xylem. I prefer to speak of a gradient of electric charge or hydration, since other factors than the H-ion concentration are involved in colloidal stability, as I have already mentioned. This theory has been substantiated in so far as it has been found that the phloem is more negatively charged and the xylem more positively or less negatively (3). It is easy to see that from a loss of charge dead cells as those of the xylem must result by a process of necrobiosis.

It is therefore very important to examine the changes in electric charge of the protoplasmic colloids and to investigate which cells are more positively and which more negatively charged. One of my students is undertaking a study of this kind.

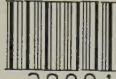
Several biologists have assumed that protoplasm shows a hysteresis which finally would lead to a loss of charge and to death. Therefore, in long-living cells there must be a force which keeps up this electric charge during the life of the cell. The source of this counteracting force is most probably the presence of the growth-hormones, which increase the hydration capacity of the protoplasm during growth and also keep it up after growth in volume is over. It has been found that no growth took place if the growing point, where those substances are manufactured, was cut off. Exactly the same thing happens if a plant, e.g., wheat, is fertilized. Before fertilization those growth-hormones diffuse into the lower parts of the plant and keep up the water- and salt-holding capacity of the vegetative parts. But as soon as fertilization has taken place, the growing-point is virtually lost for the plant and this capacity must decrease all over the plant. That means that water and salts must be lost from the vegetative parts. Indeed, I found that in wheat, which I grew under constant conditions, a sudden loss of water occurred just after flowering (1). Moreover, it is known that salts are lost from the stems and leaves after fertilization and are absorbed by the growing seeds or fruits. This is easily understood, as the young seeds form a new center of embryonic growth where new growth-hormones are manufactured. In the young seeds the water- and salt-holding capacity will increase, whereas it decreases in the rest of the plant. The salts and other foodstuffs are monopolized by the young seeds, the rest of the plant is exhausted. If we remove the seeds or fruits, we may restore the vegetative power of the plant (9), provided growing-points are present to manufacture growth-hormones for the plant. We may speak in this way of an attraction power for water and foodstuffs in the young seeds, fruits, or growing cells in general. This is the *colloid-chemical base of the life-cycle of plants*.

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